



## Application of herbal probiotics in feed on growth and blood profile of elver eels (*Anguilla bicolor* McClelland, 1844)

Harun<sup>1</sup>, Mu'amar Abdan<sup>2,\*</sup>, Suprihadi<sup>1</sup>, Fazril Saputra<sup>3</sup>, Khairunnisak<sup>1</sup>, Said Samsuli<sup>4</sup>, Rahmat Sulni<sup>3</sup>

<sup>1</sup>Abli Usaba Perikanan (AUP) Polytechnic, Aceh Campus, Aceh Besar, Aceh, 23381

<sup>2</sup>Aquaculture, Faculty of Agriculture, Gajah Putih University, Central Aceh, Aceh, 24560

<sup>3</sup>Aquaculture, Faculty of Fisheries and Marine Sciences, Teuku Umar University, West Aceh, Aceh, 23617

<sup>4</sup>Mina Mandiri Hatchery, Beutong, Nagan Raya, 23672

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### ABSTRACT

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Shortfin eel cultivation in Indonesia has shown significant development in aquaculture. However, various challenges still hinder its successful implementation. This study aims to investigate the impact of herbal probiotics on the growth and blood profile of elver eels (*Anguilla bicolor*). The research was conducted at UPR Mina Mandiri in the Beutong District of Nagan Raya Regency, from August to October 2022. This research was conducted at UPR Mina Mandiri, Beutong District, Nagan Raya Regency, from August to October 2022. A Completely Randomized Design (CRD) with five treatment levels and three replications was employed for this study. The treatments consisted of herbal probiotics added to the eels' feed: 0, 15, 20, 25, and 30 ml/kg. The data obtained from the experiment were subjected to an analysis of variance (ANOVA) to assess the significance of the results. ANOVA test results show that probiotics significantly affect survival rate, absolute weight growth, specific weight rates, feed efficiency, and the elver eel blood profile (hemoglobin, erythrocytes, and leukocytes). ( $P > 0.05$ ). Duncan's advanced test revealed significant differences in the growth parameters and blood profiles among the various treatments. Treatment B, which utilized a dosage of 15 ml/kg of feed, displayed the most favorable outcomes. It achieved a survival rate of 93.33%, absolute weight growth of 2.69 grams, a specific growth rate of 1.73%, and a feed efficiency of 48.56%. Additionally, the blood profile measurements for treatment B were as follows: hemoglobin levels ranged from 9.53 to 9.73 g/dl, erythrocyte count ranged from 1.12 to 1.23 x 10<sup>3</sup> cells/mm<sup>3</sup>, and leukocyte count ranged from 120 to 133 x 10<sup>3</sup> cells/mm<sup>3</sup>.

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### Introduction

Shortfin eels (*Anguilla* sp.) are the most commonly caught and found fish in freshwater and estuarine environments (Horváth dan Municio, 1998). According to McKinnon (2006) the life cycle of shortfin eels fish generally consists of five stadia: larvae (*Leptocephalus*), glass eel, elver eel, yellow eel, and silver eel. The high demand for eel in the international market (Asia, Japan, and European countries) and local markets cause the need for eel to increase.

Noor dan Abidin (2019), several countries in East Asia and several European countries make this commodity a favorite and classy culinary with a relatively high selling price; this makes eel a very potential commodity as an export product. In addition, the FAO (2014) data states that in terms of the price of eel in the international market, it is sold between IDR 180,000,00 – IDR 300,000,00/kg with demand reaching 130,000 tons/year, while in the local market for consumption measures range between IDR 120,000,00 – IDR 180,000,00/kg

\* Corresponding author.

Email address: [muammarabdan@gmail.com](mailto:muammarabdan@gmail.com)

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(KKP, 2011). KKP (2020) Mentioning the last two years (2019 - 2020), Indonesia has become the number one eel exporter in the world with export volumes above 10,000 tons per year (25%) of the total request for world eel exports which reaches 58,000 tons/year.

The development of eel farming has increased in Indonesia, but in practice, this fish farming is still experiencing various obstacles. Topan dan Riawan (2015) state that the pool used in eel elections will determine the success of cultivation. Another problem is the maintenance process of glass eel, which is very vulnerable to a 30-50% survival rate. In addition, the type of feed and nutritional needs in the growth process is other factors determining the success of eel farming.

Efforts to increase nutrition in feed, one of which can be done with probiotic applications, are considered to improve production performance and the survival rate of fish. Mansyur dan Tangko (2008) state that probiotics are living microorganisms that provide health benefits when given in adequate quantities; besides that, probiotics can also maintain the quality of water aquaculture.

Several research reports suggest that the implementation of probiotics can play a significant role in improving water quality, enhancing biosecurity, serving as a dietary supplement, augmenting disease resistance, increasing productivity, improving feed efficiency, and reducing production costs by decreasing the expenses associated with feed (Kesarcodi-Watson et al., 2008; Avnimelech and Kochba, 2009; Kuhn et al., 2009; Nayak, 2010; Sayes et al., 2018).

The abundance of herbal plants that are easily obtained is an important opportunity for its utilization. Viena et al. (2018) Mentioning Indonesia, a tropical country, is famous for the diversity of various herbal plants. The community uses this plant because of its nature, which can provide positive for humans and other living things (Jumiarni dan Komalasari, 2017; Haniarti et al., 2018). The natural chemical nature of herbal plants has provided a function in preventing various diseases, performing certain biological functions, and preventing insect and fungal attacks (Hidayanto, 2015).

The combination of herbal plants with probiotics (*Herbal probiotics*) can provide production and health performance in fish, and this can be seen from several studies of herbal probiotics such as tilapia fish (*Oreochromis niloticus*) (Arief et al., 2015; Arsyad dan Muharam, 2015), catfish (*Clarias gariepinus*) (Puspitasari, 2019; Abdan et al., 2022), Pomfret fish (*Colossoma macropomum*) (Saselah dan Mandeno, 2017),

depik fish (*Rasbora tawarensis*) (Frativi et al., 2018). Herbal probiotic research in eel has never been done.

The selection of herbal probiotics is superior due to their natural properties, free from synthetic chemicals and harmful residues (Ringo dan Olsen, 1999). Additionally, herbal probiotics contain active components such as antimicrobials and antioxidants, providing additional benefits to fish by enhancing immune systems and protecting against infections. The microbial diversity within herbal probiotics also exerts a synergistic effect, maintaining the microbial balance in the fish's digestive system. Moreover, the sustainable use of herbal probiotics minimizes the reliance on synthetic chemicals and reduces negative environmental impacts, making it an environmentally friendly alternative in aquaculture (Hoseinifar et al., 2018). Based on these reasons, the preference for herbal probiotics over commercial probiotics is justified as it ensures safety, offers additional benefits, provides a broader microbial diversity, and supports sustainability in fish cultivation. Therefore, this study aims to analyze the use of herbal probiotics in elver eels (*Anguilla bicolor*).

## Methods

### Time and Location

The study was conducted from August to October 2022 at the Mina Mandiri Hatchery, Beutong District, Nagan Raya Regency. Sample analysis was carried out in the Mina Mandiri laboratory.

### Materials

The tools used in this study include aquariums for fish maintenance, a shredder machine, an aerator, a syringe, fish scars, digital scales, measuring cups, pH meters, thermometers, Dissolved oxygen (DO) meters, ammonia test kit, hemacytometer, microhematocrit, Sahli tube and Erlenmeyer tube. The ingredients include Elvel eel, turmeric, fine bran, sugar, yeast, label paper, commercial feed, and EM4.

### Research design

This study uses an experimental method with a complete random design (CRD) with five treatments and three replications, namely:

- A (0 mg/l): Treatment without giving herbal probiotics
- B (15 mg/l): Herbal probiotics at a dose of 15 ml/kg of feed
- C (20 mg/l): Herbal probiotics at a dose of 20 ml/ kg of feed
- D (25 mg/l): Herbal probiotics at a dose of 25 ml/ kg of feed

- E (30 mg/l): Herbal probiotics at a dose of 30 ml/ kg of feed

## Research procedure

### *Preparation of containers and test fish*

The container used in the form of aquariums with a size of 60 x 30 x 40 cm; as many as 15 units were cleaned with detergent and then disinfected using potassium permanganate (PP) at a dose of 15 mg/l; then the aquarium was rinsed and let dry. Furthermore, the aquarium is filled with 20 liters of water. Elver eel refers to [Rahmawati \(2015\)](#), namely five individuals/liters. Before it is spread on the maintenance media, acclimatization is carried out to adjust the temperature of the maintenance media. Maintenance is carried out for 50 days, with feeding adjusted to the weight of fish every time sampling is done. The frequency of feeding three times a day is 08.00, 12.00, and 16.00 WIB. During the maintenance of water changes every morning before feeding as much water as waste. In addition, water quality observations are also carried out, including temperature, DO, ammonia, and pH.

### *The process of making probiotics*

Making herbal probiotics refers to [Abdan et al. \(2022\)](#), by inserting 5 liters of water that have been heated into a basin and 25 grams of sugar added, then followed by 500 grams of fine bran, 500 grams of fine turmeric, EM-4 200 ml, and 15 grams of yeast. For the ingredients that have been mixed, stir until well blended, then put into a closed container (5-liter capacity) and tightly closed. Closed container cans are placed in a room not exposed to sunlight; the fermentation process occurs for 7 (seven days) and is ready for use. Cover the container is opened every two days for 2-3 minutes to remove fermented gas. Before use, fermentation is filtered using a filter to separate the pulp and fermented extract, which is then mixed into the feed. The success of probiotics is characterized by the acid odor caused by fermentation. To maintain bacteria in probiotics, adding sugar is carried out as a bacterial intake, which refers to [Saselah dan Mandeno \(2017\)](#).

### *Feed enrichment process*

The feed is commercial with a protein content of 39 - 41%, fat 5%, 6%, ash 18% and water 10%. Feeding is given at the station. The feed enrichment process is carried out by refining following the dose of each treatment, and the feed is stirred and then added water to sufficient so that the feed is easily formed, and then the feed is applied.

## Parameters

### *Survival Rate (SR)*

Survival is calculated based on the equation ([Effendie, 1979](#)):

$$SR = N_t/N_0 \times 100$$

Where  $N_t$  = number of fish at the end of maintenance (Ind),  $N_0$  = number of fish at the beginning of maintenance (Ind)

### *Absolute weight growth (AWG)*

The weight gain is calculated by the formula ([Effendie, 1979](#)):

$$AWG = W_t - W_0$$

Where  $W$ : Increased Weight (gr),  $W_t$ : Biomass Weight At the end of the study (gr),  $W_0$ : biomass weight at the beginning of the study (gr)

### *Specific Growth Rate (SGR)*

The specific growth rate is the difference between each weight and length in one sampling period. The specific growth rate (SGR) can be calculated using the formula ([Marzuqi et al., 2012](#)) as follows:

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

Where SGR = specific growth rate,  $W_0$  = Average initial weight (gram),  $W_t$  = Average final weight (gr),  $t$  = time (day)

### *Feed efficiency (Fe)*

Feed efficiency is the amount of feed that fish utilize. The high value of feed efficiency shows a good level of feed use. Calculation of feed efficiency is done using the formula ([Afrianto dan Liviawaty, 2005](#)) as follows :

$$Fe = \frac{(W_t + D) - W_0}{(F)} \times 100$$

Where,  $Fe$  = feed efficiency (%),  $F$  = feed conversion (%),  $D$  = mortality (gr)

### *Hemoglobin calculation*

The calculation of hemoglobin refers to the Sahli method. Sample blood was taken using Sahli's pipette to a 20 mm<sup>3</sup> or 0.2 ml scale. Then the tip of the pipette is cleaned with tissue paper. The sample blood in the pipette was transferred to the Hb-meter tube filled with 0.1 N HCl on a scale of 10. After that, the blood is stirred with a stirring stem for 3 to 5 minutes. Furthermore, distilled water is added to the tube until the color of the blood becomes the color of the standard solution in the Hb-Meter. Hemoglobin levels are expressed in g%.

### *Erythrocyte calculation*

The calculation of several erythrocytes is measured according to Blaxhall (1972); First, blood is taken with a pipette containing a red mixer to scale 1 (pipette to measure the number of red blood cells), then add Hayem's solution to a scale so that blood is mixed evenly. The first two drops of blood solution in the pipette are discarded, then drop it on the Neubauer-type hemocytometer with a covered glass. Then, calculate the number of red blood cells with the help of a microscope with an enlargement of 400 x. Total erythrocytes are calculated in as many as four boxes and then calculated by the formula:

$$\sum \text{erythrocytes} = \text{average erythrocyte cells} \times \frac{\text{thinner}}{\text{Volume}}$$

#### Leukocyte calculations

The number of leukocytes is calculated according to Blaxhall (1972); blood samples are taken with a pipette containing a white stirrer to a scale of 0.5. Next, add the Turk's solution to a scale of 11. The pipette is swung to form the number 8 (the same as stirring for calculating the number of red blood cells) for 3-5 minutes to mix the blood evenly. Then, the first two drops of the solution in the pipette are removed, then dropped on the hemocytometer. Finally, covered with a cover glass and calculated with a 400 x magnification microscope. Total leukocytes are calculated in as many as four boxes and then calculated by the formula :

$$\sum \text{leukocytes} = \text{Average erythrocyte cells} \times \frac{\text{thinner}}{\text{Volume}}$$

## Results and Discussion

### Results

Elver eel's absolute weight growth rate for 50 days shows an increase in weight of 3.78 gr - 6.47 gr (Figure 1). ANOVA results show that applying herbal probiotics in different doses significantly affects weight growth, specific growth rates, survival, and feed efficiency. The same was also obtained on the blood profile, namely hemoglobin, leukocytes, and erythrocytes, which showed significant influence ( $P < 0,05$ ). Duncan's test results show significant differences between treatments in the growth parameters and blood profiles, with the best results obtained in treatment B (15 ml/kg of feed) (Table 1; Table 3).

### Discussion

Growth is one of the important indicators of success in fish farming which is characterized by increasing the weight of the body of the fish. Growth is related to the increase in the number and size of

cells in the fish's body so that more quality feed needs are needed (Abdan et al., 2017). Ardyati (2018) states that improving feed quality can be done by adding probiotics.

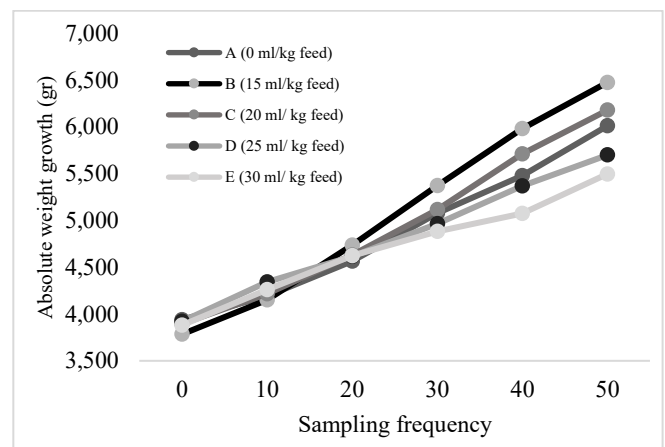


Figure 1. Elver eels growth maintained for 50 days

Based on the results of the study, it can be seen that treatment B (15 ml/kg of feed) is the best in this study. The high growth of elver eel in this study, both the growth of absolute weight and specific growth rates, refer to the role of microorganisms from herbal probiotics in increasing digestibility and efficiency of the use of nutrients in the body of the fish so that it has an impact on increasing protein retention, carbohydrate retention, and fat retention. This result is evidenced by the best feed efficiency obtained in the same treatment. Abdan et al. (2022) mention that probiotics containing bacterial microorganisms have influenced the growth and efficiency of catfish feed (*Clarias gariepinus*). The same thing also states Fuadi et al. (2019) that the presence of microorganisms originating from probiotics can support the process of feed digestibility in the fish's intestines.

The content of herbal probiotics in *Bacillus* sp. can produce enzymes that can break down complex compounds into simple compounds so that fish efficiently utilize them. The presence of enzymes produced will help hydrolyze molecules in feed, such as breaking down carbohydrates, proteins, and fat into simpler molecules, so digestion and absorption of food in the intestine occurs quickly (Putra, 2010).

The use of probiotics containing *Bacillus* sp. has also been evaluated in several types of freshwater fish and is proven to be able to increase the growth and efficiency of the use of feed, for example, in carp fish (*Cyprinus carpio*) (Suxu et al., 2011), In catfish (*Clarias gariepinus*) (Abdan et al., 2022), silver catfish (*Pangasius hypophthalmus*) (Jusadi et al., 2004), and tilapia (*Oreochromis niloticus*) (Rusdani et al., 2016; Kurniawan et al., 2019).

Aquaculture management greatly influences fish survival, including stocking dens, feed quality, water quality, and disease infection (Monalisa dan Minggawati, 2010; Diansyah et al., 2014; Wangni et al., 2019). Abdan et al. (2017) feed containing good nutrition is instrumental in maintaining survival and accelerating fish growth.

The results also showed decreased treatment C, D, and E growth. This is allegedly not as much as the balance of microbes in the digestive tract, which impacts the disruption of the fish's digestive system. The same thing was stated by Gomez-Gil et al., 2000 and Raja et al., 2015 that the negative impact of excess probiotics can result in disruption of the larvae' digestive system and fish seeds. Sya'bani et al. (2015) also state that excessive probiotics will impact the ecological Embalanches of Microbes in the Digestive Tract. In addition, other impacts can damage environmental conditions if excessively applied; for example, under normal conditions, bacteria in probiotics can be beneficial, but in abnormal

conditions will damage the environment of cultivation (Verschuere et al., 2000; Denev et al., 2009)

Fish health is a significant component in the success of aquaculture. The physiological condition of the fish will give a picture of blood chemistry both quantitatively and qualitatively. Caruso et al. (2005) also argue that blood components can indicate fish health checks. In this study, hemoglobin examination was significantly different between treatments. This indicates that probiotics can increase hemoglobin in Elver eel. Hemoglobin helps transport oxygen, carbon dioxide, and food to all body tissues. Increased hemoglobin in the blood will help increase the transportation of food and oxygen to all body tissues, which impacts the increase in fish growth. The same thing was reported by Diansyah et al. (2014) and Fekri et al. (2018) that an increase in the amount of hemoglobin is one of the factors in increasing the growth of eel fish (*Anguilla* sp.).

**Table 1.** Elver eel growth that was maintained for 50 days

Parameters	Treatments				
	A (0 ml/kg)	B (15 ml/kg)	C (20 ml/kg)	D (25 ml/kg)	E (30 ml/kg)
	<b>Growth</b>				
<i>Survival Rate (SR)(%)</i>	85,33±5,03 <sup>bc</sup>	93,33±3,05 <sup>a</sup>	87,33±3,05 <sup>ab</sup>	86,00±5,29 <sup>bc</sup>	79,33±1,15 <sup>bc</sup>
<i>Absolute weight growth (AWG) (gr)</i>	2,06±0,45 <sup>ab</sup>	2,69±0,57 <sup>a</sup>	2,09±0,33 <sup>ab</sup>	1,77±0,07 <sup>b</sup>	1,61±0,14 <sup>b</sup>
<i>Laju Pertumbuhan Spesifik (SGR (%/h)</i>	1,65±0,01 <sup>b</sup>	1,73±0,04 <sup>a</sup>	1,68±0,01 <sup>b</sup>	1,60±0,01 <sup>c</sup>	1,56±0,01 <sup>c</sup>
<i>Feed Efficiency (Fe)(%)</i>	39,20±0,27 <sup>b</sup>	48,56±0,24 <sup>a</sup>	46,07±0,34 <sup>a</sup>	38,47±0,36 <sup>b</sup>	37,80±0,44 <sup>b</sup>

Note: the same superscript on the same row is not significantly different (P<0,05)

**Table 2.** Water quality maintained for 50 days

Parameters	Treatments					Optimal parameters According to (Diansyah et al., 2014)
	A (0 ml/kg)	B (15 ml/kg)	C (20 ml/kg)	D (25 ml/kg)	E (30 ml/kg)	
<i>Temperature °C</i>	24,2 – 29,1	25,1 – 29,8	24,7 – 30,2	24,3– 29,9	25,2 – 29,1	27 – 31
<i>pH</i>	6,4 – 7,9	6,5 – 8,1	6,4 – 8,0	6,7 – 7,8	6,4 – 8,2	6,8 – 8
<i>Ammonia (NH<sub>3</sub>) (mg/L)</i>	0,01	0,01	0,01	0,01	0,01	0,007 – 0,01
<i>Dissolved Oxygen (DO) (mg/L)</i>	4 - 6	4 - 6	4 - 6	4 - 6	4 - 6	4,5 – 8,6

**Table 3.** Elver Eel Blood Profile Maintained for 50 days

Blood profile	Treatments	Times	
		Days 10	Days 50
<i>Erythrocyte (x10<sup>6</sup> cell/mm<sup>3</sup>)</i>	A (0 ml/kg)	1,25±0,12 <sup>a</sup>	1,36±0,12 <sup>b</sup>
	B (15 ml/kg)	1,12±0,11 <sup>a</sup>	1,23±0,09 <sup>a</sup>
	C (20 ml/kg)	1,15±0,10 <sup>a</sup>	1,24±0,10 <sup>a</sup>
	D (25 ml/kg)	1,24±0,12 <sup>a</sup>	1,54±0,12 <sup>c</sup>
	E (30 ml/kg)	1,17±0,12 <sup>a</sup>	1,61±0,10 <sup>c</sup>

<i>Leukocyte</i> ( $\times 10^3$ cell/mm <sup>3</sup> )	A (0 ml/kg)	119,62 $\pm$ 0,12 <sup>a</sup>	132,35 $\pm$ 0,13 <sup>a</sup>
	B (15 ml/kg)	120,30 $\pm$ 0,10 <sup>b</sup>	133,27 $\pm$ 0,12 <sup>b</sup>
	C (20 ml/kg)	121,75 $\pm$ 0,13 <sup>c</sup>	129,35 $\pm$ 0,12 <sup>c</sup>
	D (25 ml/kg)	116,39 $\pm$ 0,11 <sup>e</sup>	123,95 $\pm$ 0,11 <sup>d</sup>
	E (30 ml/kg)	117,39 $\pm$ 0,12 <sup>d</sup>	122,38 $\pm$ 0,13 <sup>c</sup>
<i>Hemoglobin</i> (g/dL)	A (0 ml/kg)	9,29 $\pm$ 0,09 <sup>ab</sup>	9,68 $\pm$ 0,10 <sup>a</sup>
	B (15 ml/kg)	9,53 $\pm$ 0,10 <sup>a</sup>	9,73 $\pm$ 0,15 <sup>a</sup>
	C (20 ml/kg)	9,48 $\pm$ 0,09 <sup>b</sup>	9,61 $\pm$ 0,11 <sup>ab</sup>
	D (25 ml/kg)	9,14 $\pm$ 0,08 <sup>c</sup>	9,33 $\pm$ 0,09 <sup>c</sup>
	E (30 ml/kg)	9,09 $\pm$ 0,14 <sup>c</sup>	9,44 $\pm$ 0,16 <sup>bc</sup>

Note: the same superscript on the same row is not significantly different ( $P < 0,05$ )

Erythrocytes are the most number of cells. High erythrocytes in fish indicate stress in fish (Gboire et al., 2006). The range of red blood cells (erythrocytes) in this study is still in the normal range as referred to by Şahan et al. (2007), namely, the number of normal erythrocytes in eel fish ranges from 1.2-1.3 $\times 10^6$  cells/mm<sup>3</sup>. This shows that the number of fish elver erythrocytes in this study is still in the normal range of 1.12 - 1.63  $\times 10^3$ .

One component of blood that acts as a non-specific body defense is leukocytes. Leukocytes will localize and prevent pathogens through the process of phagocytosis. According to Lagler et al. (1977), normal leukocytes for elver eel are 20  $\times 10^3$  - 150  $\times 10^3$ . This study's results indicate that the leukocyte range is still in the normal range of 116  $\times 10^3$  - 133  $\times 10^3$ .

Another factor that determines the success of cultivation is water quality. Water quality is a determining factor that directly affects the survival and growth of fish. Therefore, water quality management is needed that can support cultivation activities. The results showed that the range of water quality is still in the normal range for the growth of elver eel; this shows that the role of probiotics, besides being able to improve the performance of fish production and health, also able to maintain water quality (Table 2). According to Harianto et al. (2020) and Diansyah et al. (2014), The range of good water quality in the maintenance of elver eel is 27 - 31 °C, pH 6.6 - 8.1, DO > 3 mg/l and ammonia <0.01 mg/l.

## Conclusion

The application of herbal probiotics in feeding with different doses significantly affects the growth and blood profile of elver eels ( $P < 0,05$ ), and the results of further tests are significantly different between the treatment of each parameter. Treatment B (15 ml/kg of feed) is the best treatment in this

study. So herbal probiotics are recommended for 15 ml/kg of feed in maintaining elver eels.

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