Exploring the potential of endophytic bacteria isolated from *Sargassum* sp.: Enhancing growth and non-specific immune response of cobia *Rachycentron canadum* (Linnaeus, 1766)

[1,2]Fittrie M. Pratiwy, [2]Muhammad I. Sumantri, [3]Suryadi Saputra, [1,2]Rosidah, [1,2]Walim Lili, [1,2]Iskandar, [4]Thallita N. Faddilah, [5]Rafidah Othman

[1] Department of Fisheries, Faculty of Fishery and Marine Science, Universitas Padjadjaran, West Java, Indonesia; [2] Study Program of Fisheries Science, Faculty of Fishery and Marine Science, Universitas Padjadjaran, West Java, Indonesia; [3] Research Center of Fisheries, National Research and Innovation Agency, Indonesia; [4] Study Program of Marine Conservation, Faculty of Fishery and Marine Science, Universitas Padjadjaran, West Java, Indonesia; [5] Borneo Marine Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia. Corresponding author: F. M. Pratiwy, fittrie.pratiwy@unpad.ac.id

Abstract. This study aimed to investigate the effects of adding endophytic bacterial isolates and *Sargassum* sp. algae extract to enhance the growth performance and non-specific immune response of cobia (*Rachycentron canadum*) fry. The research employed a Completely Randomized Design (CRD) experimental method with three treatments and five replications. The treatments consisted of: (A) commercial feed without any additives, (B) commercial feed supplemented with 15 mL of *Sargassum* sp. extract, and (C) commercial feed supplemented with 15 mL of endophytic bacterial isolates from *Sargassum* sp. at a concentration of 108 CFU mL^{-1}. The fish fry were reared for 30 days, followed by a 14-day challenge test with *Vibrio alginolyticus* bacteria at a density of 107 CFU mL^{-1}, using the intraperitoneal (IP) injection method. Parameters observed included absolute weight gain, absolute length gain, specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency (FE), total erythrocyte count, total leukocyte count, leukocyte differential, clinical symptoms, survival rate, and water quality. Data on absolute weight gain, absolute length gain, SGR, FCR, FE, total erythrocyte count, total leukocyte count, and survival rate were analyzed using ANOVA with a 95% confidence level, followed by Duncan's post hoc test for significant differences. Leukocyte differentials, clinical symptoms, and water quality data were analyzed descriptively. The main findings revealed that the addition of endophytic bacterial isolates and *Sargassum* sp. algae extract could enhance the growth and non-specific immune response of kobia fry. Treatment C proved to be the most effective, as evidenced by the increased values of absolute weight gain (35.72 g), absolute length gain (11 cm), SGR (3.17%), FCR (1.19%), FE (84.13%), total erythrocyte count (2.89×106 mm^{-3}), total leukocyte count (38.56×103 mm^{-3}), and leukocyte differential percentages (lymphocytes 82.2%, monocytes 4.4%, neutrophils 8.8%), as well as the fastest recovery time (7 days) after the challenge test, with the highest survival rate of 90.7%.

Key Words: aquaculture, cobia fry, feed additives, immunostimulant, vibrio.

Introduction. Cobia (*Rachycentron canadum*) is found across tropical and subtropical waters worldwide, offering significant potential for aquaculture due to its rapid growth rate and commercial appeal (Ademola 2024). However, the industry faces some threats, including viral, bacterial, and parasitic diseases (Lavilla-Pitogo et al 1990; Rajan et al 2001), particularly in regions where cobia aquaculture is established. Bacterial disease is a prevalent disease in marine aquaculture. Specifically in vibriosis, it impacts numerous species of economically important aquatic organisms globally, leading to significant challenges for the industry (Sem et al 2023). Vibriosis, caused by various *Vibrio* species, emerges as a primary disease affecting marine fish in both salt and brackish waters, resulting in substantial economic losses within the mariculture industry and impacting numerous cultured and wild fish and shellfish species (Mohamad et al 2020). Disease outbreaks are often linked to increased water temperatures in late summer, especially in shallow and near-shore waters. While *Vibrio alginolyticus* was traditionally considered part of the normal marine flora (Chart 2012), it has been implicated as a pathogen affecting several marine fish species, including silver sea bream (*Pagrus auratus*) (Rameshkumar et al 2017), cobia (*Rachycentron canadum*) (Rajan et al 2001; Rameshkumar et al 2014), grouper (*Epinephelus* sp.) (Glamuzina & Rimmer 2022), and Asian seabass (*Lates calcarifer*) (Islam et al 2024).

Diseases in fish can be caused by various infectious agents such as viruses, bacteria, fungi, and parasites. Fish that present wounds, nutrient deficiencies, and poor water quality may experience decreased immunity, potentially leading to disease due to the imbalance between host factors, pathogens, and the environment (Putra et al 2018). Among the diseases most commonly affecting cobia, vibriosis caused by *Vibrio* sp. bacteria is notable, leading to hemorrhage, loss of appetite, and mass mortality (Mohammed et al 2021). Disease prevention methods in fish can be accomplished through oral or immersion vaccination and the administration of immunostimulants (Zhao et al 2019).

Immunostimulant substances can be derived from chemicals such as vitamins, minerals, and natural materials including plants and animals. Extracts from seaweed *Sargassum* spp. containing bioactive compounds have the potential to enhance immune responses in fish (Pratiwy & Pratiwi 2020). The use of vaccines is relatively more expensive and specific, targeting the prevention of specific disease agents, whereas the use of plant-derived immunostimulants is relatively cheaper and can be used for the prevention of various disease agents in a non-specific manner (Permatasari et al 2019).

Research conducted by Sujatha et al (2019) demonstrated that *Sargassum swartzii* seaweed extract contains bioactive compounds such as tannins, terpenoids, flavonoids, saponins, and phenols, showing antibacterial activity against pathogenic bacteria *Staphylococcus aureus*, *Aeromonas hydrophila, Pseudomonas aeruginosa, and Vibrio vulnificus*. Another study on *Sargassum polycystum* seaweed extract also revealed bioactive compounds such as alkaloids, flavonoids, saponins, steroids, and terpenoids, showing antibacterial activity against *S. aureus* (Riwanti et al 2021).

Endophytic bacteria are a type of bacteria whose life cycle largely involves interacting with host plants without causing disturbance or damage. Bioactive compounds produced by these endophytic bacteria have potential as antibacterials against several pathogenic bacteria attacking the host (Hasiani et al 2015). Endophytic bacteria are easily cultivable microorganisms with short life cycles capable of producing bioactive compounds in large quantities in a short time, thus holding great potential for utilization in aquaculture (Sukmawaty et al 2016).

Research on the benefits of active ingredients in seaweed extracts conducted by Kasanah et al (2019) showed that extracts from red algae *Gracilaria edulis* contain active ingredients that can potentially enhance fish immune responses against pathogenic bacteria such as *Vibrio* spp. and *Aeromonas hydrophila* at doses adjusted to fish species, size, and age. Kusumaningrum (2007) explained that immunostimulant substances from brown algae extracts contain active compounds such as phenols and flavonoids with antibacterial activity. Research conducted on *Sargassum sabrepandum* showed the presence of endophytic bacteria *Bacillus sp*. with antibacterial activity against pathogens *S. aureus* and *P. aeruginosa* (Ahmed et al 2016). Therefore, based on the previous studies, the aim of this study is to evaluate the potential of *Sargassum* sp. extracts and endophytic bacteria isolated from *Sargassum* sp. as immunostimulants to enhance growth performance and non-specific immune response in cobia fry, thereby improving their resilience against diseases caused by *Vibrio* sp.

Material and Method

Method. The main research was conducted at the Center for Marine Aquaculture (BBPBL) Lampung from February to March 2022. Preliminary research was previously conducted from June to October 2021 at the Chemistry Laboratory, Graduate Building D6, Faculty of Mathematics and Natural Sciences (FMIPA), Singaperbangsa Unpad, Bandung, the Biology Laboratory, FMIPA, Universitas Padjadjaran, Jatinangor, and PT Genetika Science, Tangerang. The preliminary research, conducted in collaboration with other research teams, aimed to identify the best algae extracts and endophytic bacteria to be used in the main research. The research method used is the experimental method with a completely randomized design (CRD), with 3 treatments and 5 replications. The treatments used are *Sargassum* sp. extract and endophytic bacteria, which were added to feed at the following doses: A - control (no addition); B - *Sargassum* sp. algae extract at 15 mL kg^{-1}; C - endophytic bacteria isolated from *Sargassum* sp. at 15 mL kg^{-1}.

The CRD utilized a linear model (Gaspersz 1991), with the following calculation formula:

$$YI = (\mu + ʇi + \epsilon ij)$$

Where: Yi - observation result on the i-th observation and i-th replication; μ - general mean value; μi - effect of treatment i; ϵij - random factor effect of treatment i and replication j.

After 30 days of rearing, fish were challenged by infection with *Vibrio* sp., with the immersion method. The bacterial solution was put into each aquarium (10 mL). Observations after the challenge test were carried out for 14 days.

Preparation of test feed. Commercial marine fish pellets for juvenile fish, with a minimum crude protein content of 40% were used as control for each treatment feeds. The endophytic bacteria used were *Pseudomonas* sp. isolated from *Sargassum* sp., which have been diluted to 10^3. The endophytic bacteria isolates were measured to obtain a density value up to 10^8 CFU mL^{-1}. The *Sargassum* sp. extract and endophytic bacteria were added into a spray containing water and a binder consisting egg white (2% of the feed weight) (Rafsyanzani & Hidayatullah 2016). This mixture was sprayed onto the feed while stirring until it was evenly distributed. In the control treatment, egg white was also added in the same amount, without the addition of any extract or bacteria. The feed mixing was performed daily throughout the fish maintenance period.

Preparation of test fish and maintenance of test fish. The cobia used were 45 days old, with a weight of 6±3.2 g and a length of 8±1.5 cm, obtained from the hatchery at the Lampung Marine Aquaculture Research Institute (BBPBL) in Lampung, Indonesia. Before stocking, the fish were graded to obtain uniform sizes, and their health condition was checked before treatments were applied. The fish were stocked at a density of 15 individuals per cage (1.25 m^3). A concrete pond with a maximum volume capacity of 4 tons of seawater was equipped with 15 hapa nets, each measuring 0.5x0.5x0.5 m^3, which were used as containers for the cultivation of cobia fry. Before use, the ponds were cleaned and disinfected. Subsequently, the ponds were dried and filled up to 70% of their volume, with two aeration points in each pond. The ponds were equipped with an inlet at the top and an outlet at the bottom, including a drainage pipe. The test fish were maintained for 22 days. Feeding was carried out three times a day in the morning at 07:00, at noon at 12:00, and in the evening at 17:00 WIB ad-libitum, with a minimum total feed at 5% of the fish biomass per treatment. Any remaining feed was removed by siphoning, and water was partially replaced by 50-70% once a day in the morning.

Clinical symptoms after infection. Bacterial infection was carried out using an intramuscular injection method with a density of 1.5x107 CFU mL^{-1}. Pathogenic bacteria were injected at a concentration of 0.1 mL per fish. Macroscopic clinical symptoms observed were changes in body surface damage, response to shock and response to feed. The damage of body surface was observed after 48 hours of experimental period. The sampling time was 4, 6, 12, 24, 32, and 48 h.

Cobia immunity. Total erythrocytes and total leukocytes cell count served as an indicator of fish health during the experimental treatment period. The procedure for obtaining red blood cells and total leukocytes was based on Blaxhall & Daisley (1973). The test fish were removed from the aquarium and an incision was made at the base of the caudal fin using a scalpel.

Water quality parameters. The water quality parameters measured include temperature, pH, salinity, dissolved oxygen (DO), total ammonia, and total nitrite. In situ water quality parameters included temperature, pH, salinity, and DO. Parameters tested in the laboratory according to the SNI 06-6989.11-2004 standards included total ammonia and total nitrite. Observations of all parameters was conducted every 20 days.

Statistical analysis. The absolute weight growth (H), absolute length growth (L), specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency (FE), total erythrocytes, total leukocytes, and survival rate (SR) data obtained were analyzed using analysis of variance (ANOVA) with a confidence level of 95%. However, if there was a significant difference observed among treatments, the post-hoc Duncan test was used further (Gaspersz 1991). The data on leukocyte differentials was determined after Amlacher (1970). Mean time to death (MTD), and water quality were analyzed descriptively.

Results

Fish growth rate. The results of the data collection on the cobia growth parameters are presented in Table 1.

Table 1

Fish growth data based on the utilization of *Sargassum* extract and endophytic bacteria

Observation parameters	*Treatment*			*Standard (BBPBL 2020)*
	A	*B*	*C*	
Mean final fry weight (g)	33.84±0.87	38.38±0.55	40.24±0.77	Fry age 60-70 days (weight ±25-33 g)
Absolute weight growth (g)	28.12±0.44[a]	33.36±0.44[b]	35.72±0.66[c]	
Average final fry length (cm)	17.82±0.77	19.24±1.48	21.02±1.03	Fry age 60-70 days (length ±17-20 cm)
Absolute length growth (cm)	8.14±0.50[a]	9.92±1.33[b]	11±0.86[c]	
SGR (% ind days^{-1})	2.58±0.08[a]	2.95±0.07[b]	3.17±0.07[c]	Weight growth rate & daily length is 2-3 %
FCR (%)	1.27±0.02[a]	1.22±0.02[b]	1.19±0.01[c]	FCR ranges between 1.18-1.45
FE (%)	78.48±1.01[a]	82.00±1.41[b]	84.13±0.86[c]	%FE ranges between 75-82.12%
SR (%)	100±0.00	100±0.00	100±0.00	Fry aged >60 days have an SR above 90%
FR (%)	5.6-6.0	5.6-6.8	5.4-6.6	FR range from 6-8%
TFC (g)	717±17.33[a]	814±16.14[b]	849±23.93[c]	

Note: FCR - feed conversion ratio; FE - feed efficiency (%); FR - feeding rate (%); SGR - specific growth rate (%); SR - survival rate (%); TFC - total feed consumption (g).

Figure 1 indicates that the absolute weight growth of fish in all treatments exhibits varying values. Treatment A (control) produced the lowest absolute weight growth value, 28.12±0.44 g, while Treatment C produced the highest absolute weight growth value, 35.72±0.66 g.

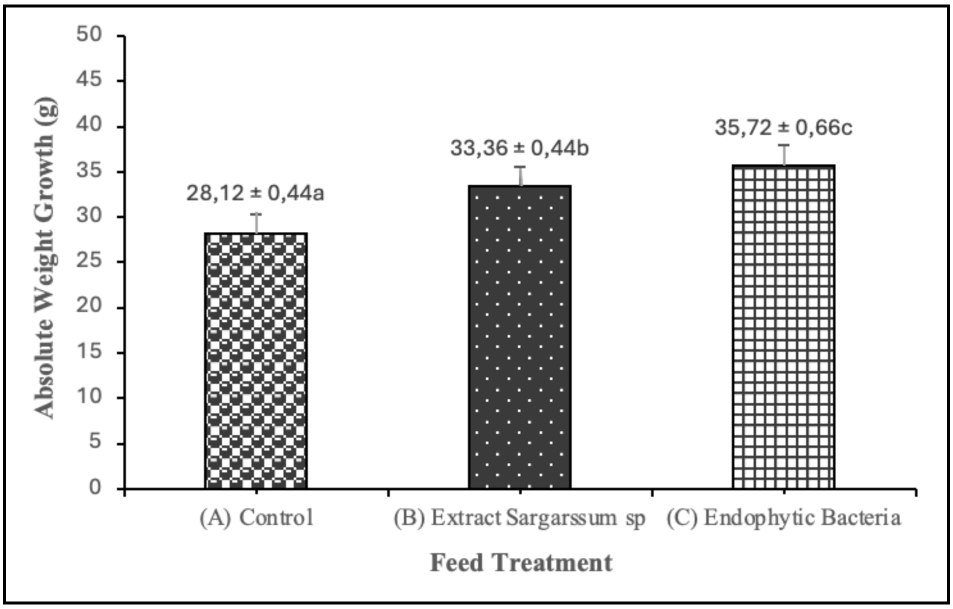

Figure 1. Absolute weight growth of cobia (*Rachycentron canadum*) fry under 3 different treatments: A - control feed; B - feed with extract of *Sargassum* sp.; and C - feed with endophytic bacteria. Different letters above bars show significant differences p<0.05.

The final length of cobia fry subjected to treatment C gave optimal results with an average length of 21±1.03 cm, representing a 110% increase from the initial length of the fish, which was 10 cm (Figure 2). In treatment B, there was a 106% increase in the length of cobia fry. This showed that the improvements observed in treatments B and C were relatively comparable in contrast to the control treatment, which only exhibited an 84% increase in length.

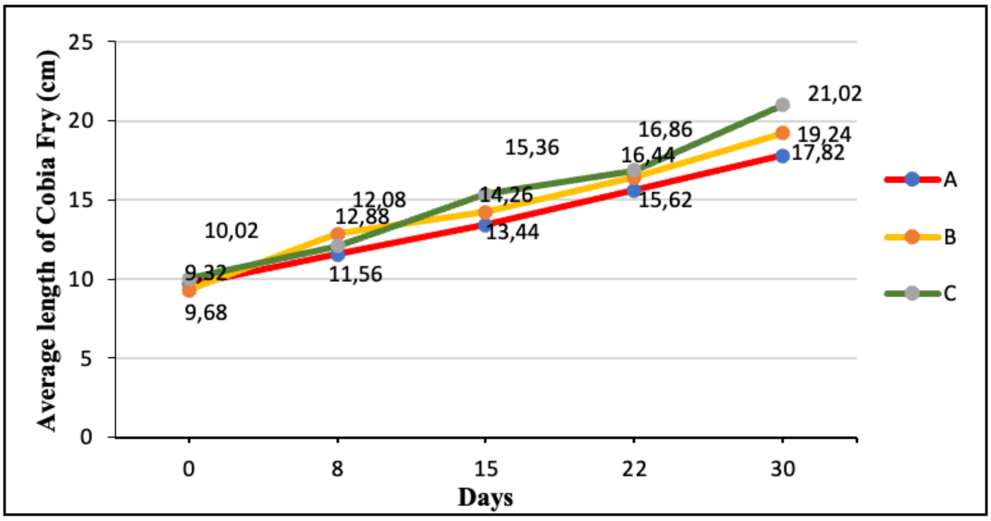

Figure 2. Average length growth cobia (*Rachycentron canadum*) fry.

Specific growth rate (SGR). SGR is a growth parameter that explains the percentage of fish growth per day. Factors that influenced the SGR include the quantity of fish feed, the fish meal level, which closely corresponds to the feeding rate, and the interaction with nutrient absorption (feed acceptability) by the fish (Li et al 2006). The value of the daily growth rate is closely related to absolute weight growth, signifying that optimal increases in fish weight within a specific time unit can elevate the SGR value. The fry stage is considered the most opportune phase for evaluating the SGR value. This is attributed to the fact that the SGR curve during the fry stage maintains an exponential form when compared with the SGR value in the adult fish stage, which exhibits a linear pattern (Lugert et al 2016).

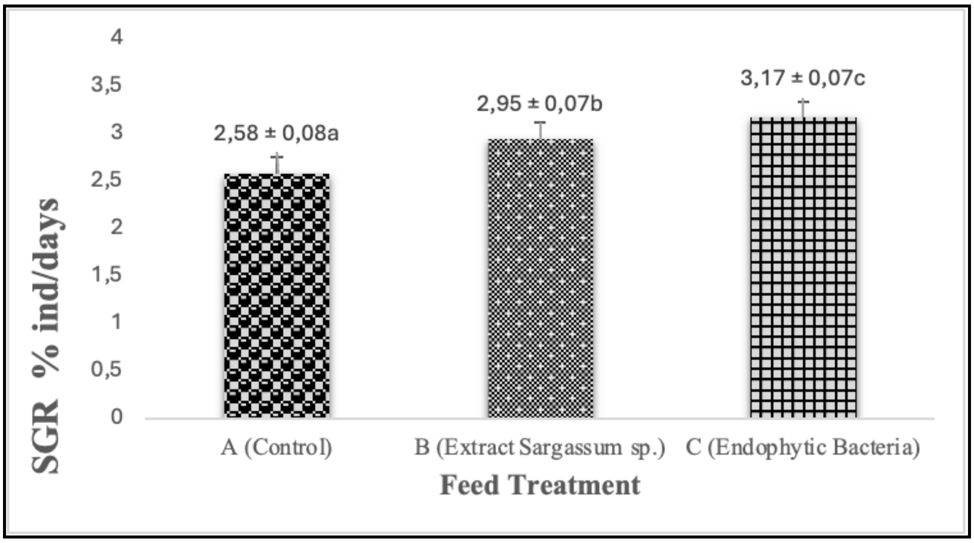

Figure 3. Specific growth rate (SGR) of cobia (*Rachycentron canadum*) fry under 3 different treatments: A - control feed; B - feed with *Sargassum* sp. extract, and C - feed with endophytic bacteria. Different letters above bars show significant differences $p<0.05$.

Feed conversion ratio (FCR). Based on Figure 4, treatment C produced the lowest FCR value, at 1.19%, indicating that the use of feed containing endophytic bacterial isolates is the most effective in enhancing the biomass of cobia fry. FCR in treatment B was not significantly different from that of treatment C, but significantly different from that of treatment A ($p<0.05$).

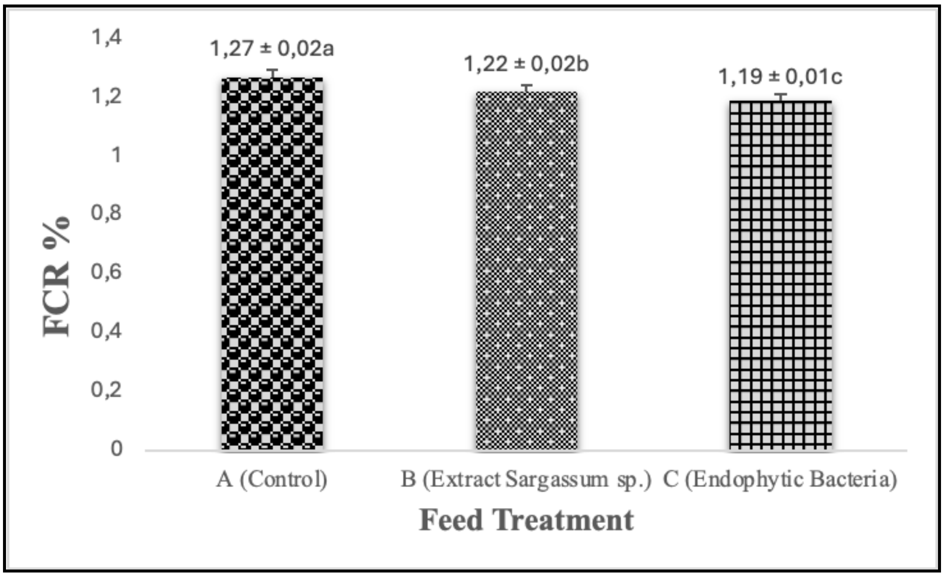

Figure 4. Feed conversion ratio (FCR) of cobia (*Rachycentron canadum*) fry under 3 different treatments: A - control feed; B - feed with extract of *Sargassum* sp.; and C - feed with endophytic bacteria. Different letters above bars show significant differences $p<0.05$.

Feed efficiency (FE). According to Figure 5, treatment A produced the lowest FE value compared to treatments B and C, with a value of 78.48%. Meanwhile, treatments B and C showed a significant increase in FE values ($p<0.05$).

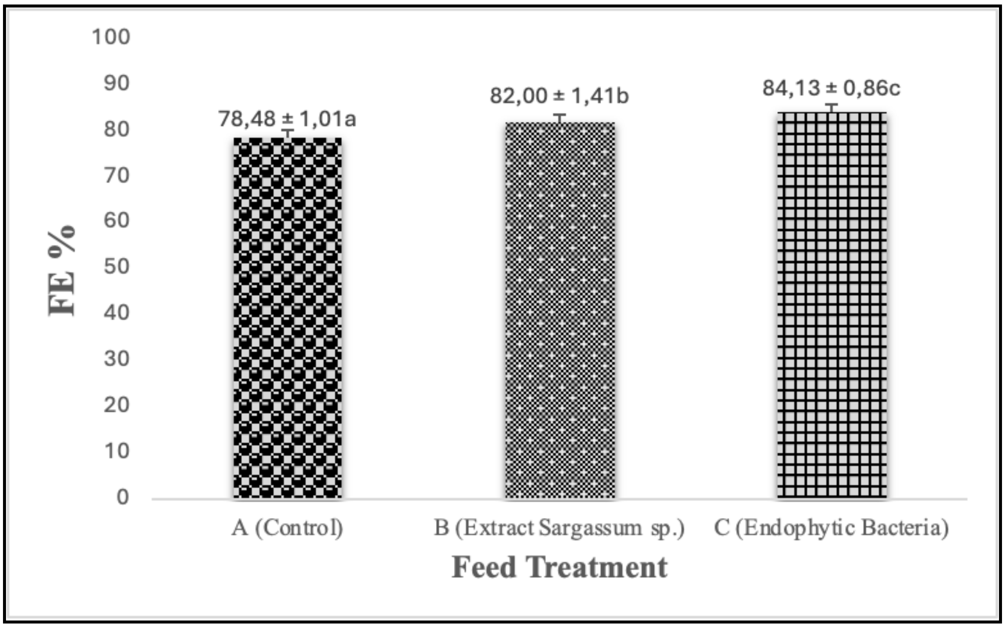

Figure 5. Feed efficiency (FE) of cobia (*Rachycentron canadum*) fry under 3 different treatments: A - control feed; B - feed with extract of *Sargassum* sp.; and C - feed with endophytic bacteria. Different letters above bars show significant differences ($p<0.05$).

FE in treatment B was not significantly different from that of treatment C, but significantly different from that of treatment A. Treatment C proved to be optimal, recording an FE value of 84.13%. Treatment C allows fish to utilize the feed more efficiently, providing more energy for growth compared to treatments A and B. Optimal growth can occur when the consumed feed has good efficiency, resulting in greater energy availability. Fish growth heavily relies on the energy obtained from feed, with needs for metabolism, hormonal systems, and other body activities. Hence, a surplus of energy is required for fish growth (Heinsbroek & Kreuger 1992).

The FE results for treatments A and B fall within the normal range, while the FE in treatment C was slightly higher than the standard FE for cobia fry, ranging between 75-82% (BBPBL 2020). This suggests that a higher FCR value signifies better feed management to avoid waste of feed and the production costs incurred (Besson et al 2016).

Immunity results (challenge test)

Erythrocytes. The average red blood cell count before treatment ranged from 2.49×10^6–2.63×10^6 cells mm^{-3}. After treatment, there was a significant increase in the number of erythrocytes in treatments B and C, which ranged from 2.89×10^6-2.92×10^6 cells mm^{-3}, while treatment A experienced a slight decrease in the number of erythrocytes, 2.5×10^6 cells mm^{-3} (Figure 6). The highest number of erythrocytes in cobia fry after treatment was in treatment B, namely 2.92×10^6 cells mm^{-3}. However, this increase is within the normal range, signifying that the juvenile cobia are in a healthy and non-stressed condition. An excessive increase in red blood cell count beyond the normal range may indicate stress due to environmental changes or disruptions in the metabolic system (Cui et al 2010).

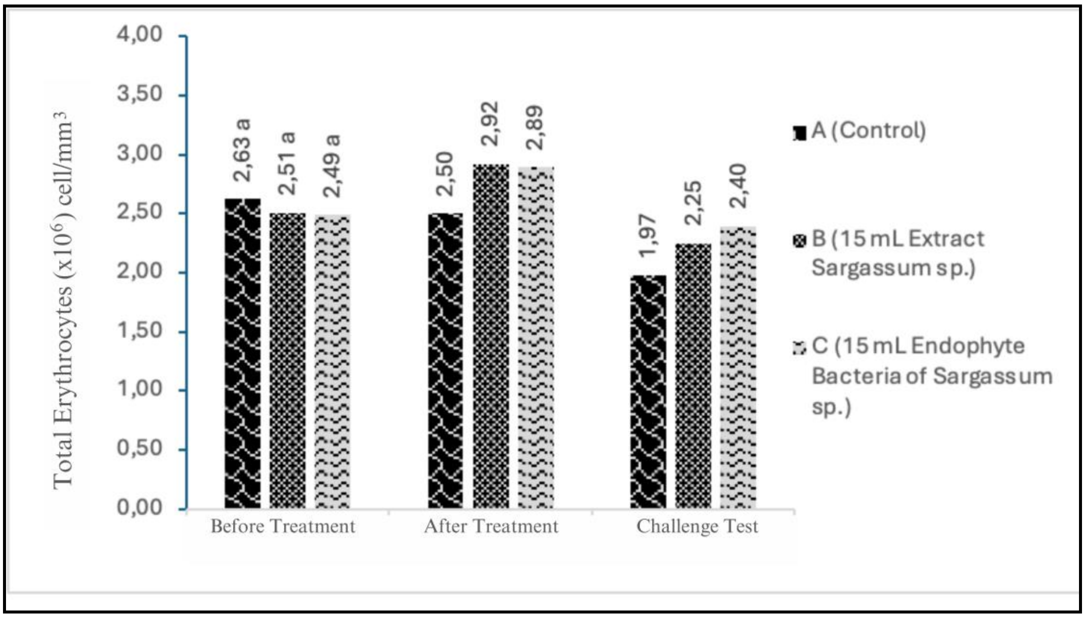

Figure 6. Red blood cell count of cobia (*Rachycentron canadum*) fry during research. Control (A), feed with 15mL extract Sargassum (B), feed with 15 mL endophyte bacteria of *Sargassum* (C).

The number of erythrocytes in cobia fry after being infected with *Vibrio alginolyticus* bacteria had significant differences depending on previous treatments. Treatment A produced significantly different results from treatments B and C, while the number of erythrocytes in treatment B was not significantly different from that in treatment C. The highest erythrocyte value was obtained in treatment C, at 2.39×10^6 cells mm^{-3}, while treatment A produced the lowest erythrocyte value, below the normal limit of 1.97×10^6 cells mm^{-3} (Table 2). Cui et al (2010) stated that the average number of erythrocytes count in healthy cobia fry ranges from $2.1\text{x}10^6$–$4.1\text{x}10^6$ cells mm^{-3}.

Table 2

Percentage of increase/decrease in total erythrocytes during research

Treatments	*Averages of total erythrocytes numbers (cell mm^{-3})*			*Changing of erythrocytes numbers (%)*	
	Before treatments	*After treatments*	*Challenge test*	*After treatments*	*After challenge test*
A (control)	2.63	2.50	1.97	-4.72%	-21.10%
B (15 mL extract of *Sargassum* sp.)	2.51	2.92	2.25	16.60%	-22.93%
C (15 mL endophyte bacteria from *Sargassum* sp.)	2.49	2.89	2.40	16.22%	-17.14%

Note: (-) shows a decrease in total of erythrocytes.

Leukocytes. The average number of leukocytes count before treatments ranged from $29.08\text{x}10^3$ to $32.16\ \text{x}10^3$ cells mm^{-3}. After treatment, an increase in the number of leukocytes count was evident in treatments B and C, ranging from $38.09\ \text{x}10^3$ to $38.56\text{x}10^3$ cells mm^{-3}, while the control experienced a slight increase in the number of leukocytes, up to $30.69\text{x}10^3$ cells mm^{-3} (Figure 7). Thus, the bioactive ingredients from endophytic bacterial isolates and *Sargassum* extract can increase leukocyte levels in the fish's body. Pratiwy & Pratiwi (2020) explain that *Sargassum* extract added at a concentration of 15 g kg^{-1} of feed administered to catfish (*Clarias sp.*) is capable of helping the immune system, indicated by an increase in the production of leukocytes and erythrocytes, the extract containing several active substances (alkaloids, phenols, and triterpenoids) and polysaccharides (fucoidan).

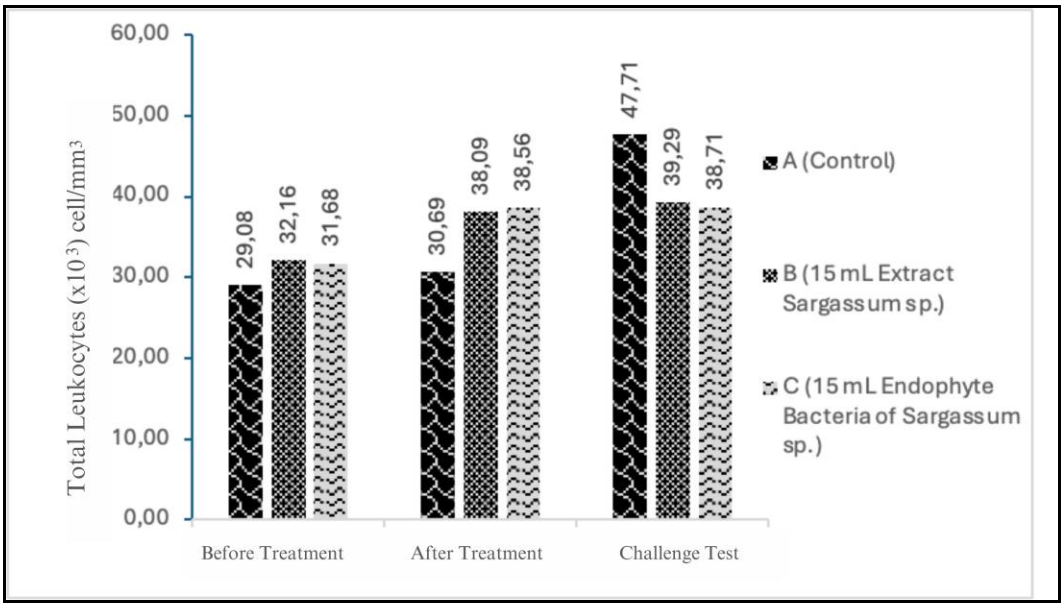

Figure 7. Number of leukocytes in cobia (*Rachycentron canadum*) fry during the research.

There was a significant change in the number of leukocytes in cobia fry after being infected with *V. alginolyticus*. The number of leukocytes in fish from treatment A was significantly different from that of treatments B and C, while the number of leukocytes in fish from treatment B was not significantly different from that of treatment C. The highest leukocyte value was obtained in treatment A, at 47.7×10^3 cells mm^{-3}, followed by treatments B and C, with 39.29×10^3 and $38.71x10^3$ cells mm^{-3}, respectively (Table 3). The number of leukocytes in treatment A exceeded the normal limit for the number of leukocytes in cobia fry, while treatments B and C experienced a trend of decreasing leukocyte numbers, but within normal limits. The number of leukocytes in healthy cobia ranges from $28.6x10^3$ to $37.97x10^3$ cells mm^{-3} (Amenyogbe et al 2022). A significant increase in leukocytes indicates that the fish is being infected by a pathogen.

Table 3

Total leukocytes during research

Treatments	*Averages of total leukocytes (cell mm^{-3})*			*Increase in leukocytes (%)*	
	Before treatment	*After treatment*	*Challenge test*	*After treatment*	*After challenge test*
A (Control)	29075	30685	47705	5.54%	55.47%
B (15 mL extract of *Sargassum* sp.)	32160	38090	39290	18.44%	3.15%
C (15 mL endophyte bacteria from *Sargassum* sp.)	31680	38560	38710	21.72%	0.39%

Differential leukocytes

Lymphocytes. Lymphocytes are the primary indicator of the formation of the body's immunity through humoral and cellular mechanisms (adaptive immune system), which function to recognize antigens, produce antibodies and destroy foreign substances that enter the body (Rojo-Cebreros et al 2018). Figure 8 shows that the percentage of lymphocytes before treatment ranged from 73-76%. After treatment, there was a significant increase in treatments B and C, namely 85% and 87%, respectively, while treatment A experienced a decrease to 73%.

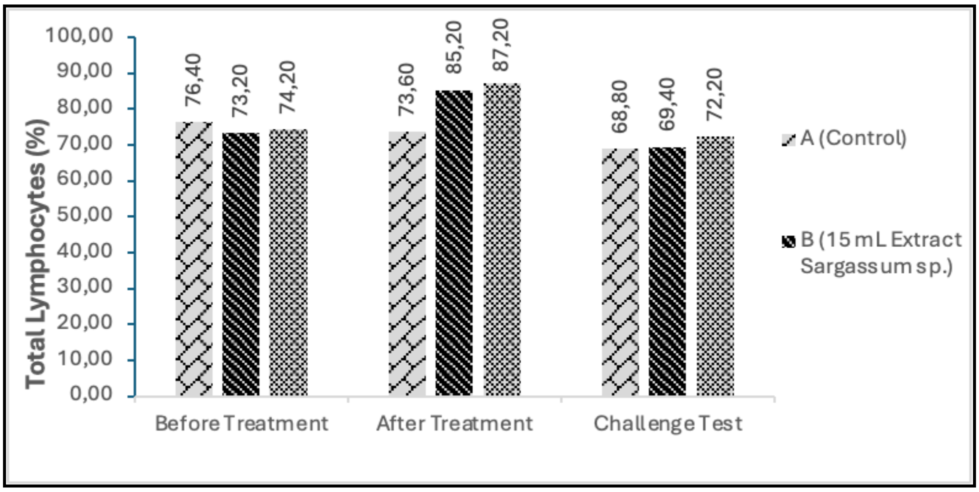

Figure 8. Lymphocytes in cobia (*Rachycentron canadum*) fry during research.

An increase in lymphocyte count was observed after the fry were treated with feeds B and C, as illustrated in Figure 8. This increase was attributed to the addition of endophytic bacterial isolates and *Sargassum* extracts, which contain various bioactive compounds acting as immunostimulants. The percentage of lymphocyte numbers after the challenge test showed a decrease in all treatments.

Monocytes. Monocytes represent one of the differentiated forms of leukocytes with phagocytic functions against antigens or foreign substances that successfully enter the body. Monocytes become active following induction by neutrophils and lymphocytes when an infection or inflammation occurs. The percentage of monocytes before treatment ranged from 4.2 to 4.6% (Figure 9). The addition of endophytic bacterial isolates and *Sargassum sp* extract did not affect the percentage of monocytes in cobia fry. According to Pratiwy & Pratiwi (2020), extract of *Sargassum* sp. provides effective results in increasing erythrocytes and lymphocytes, but does not have a significant effect on the percentage of monocytes and neutrophils. According to BBPBL (2020), the number of monocytes in healthy cobia ranges from 1.3-5.3%.

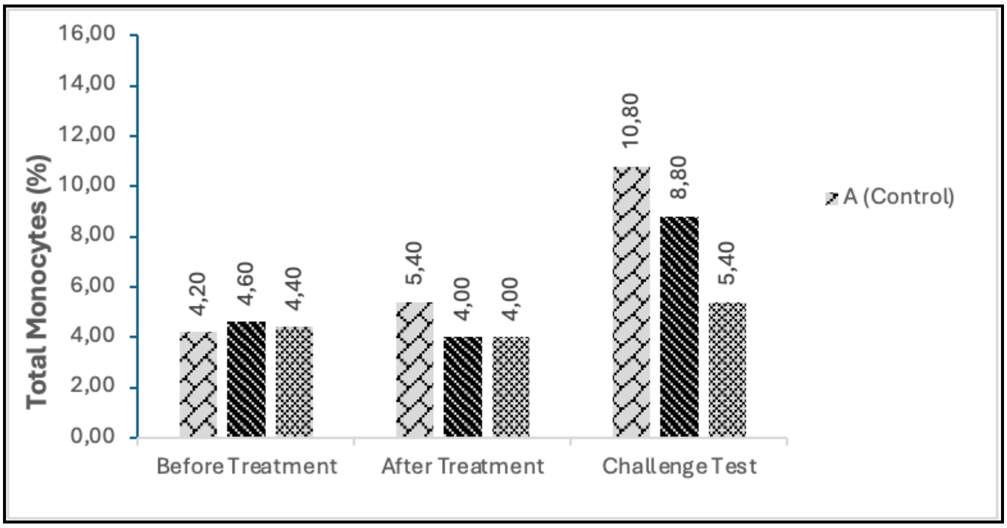

Figure 9. Percentage of monocytes in cobia (*Rachycentron canadum*) fry during research.

The percentage of monocytes after the challenge test indicates a significant increasing trend in all treatments A, B and C, namely 10.80%, 8.80% and 5.40%, respectively. The highest increase in monocytes was obtained in the control, while the lowest increase was in treatment C. The higher increase in the percentage of monocytes indicates the high virulence in the fish, meaning that more bacteria or pathogens enter the body.

Neutrophils. Neutrophils are the first type of leukocyte cells from the immune system to respond to infections and inflammations caused by bacteria or other foreign substances. Neutrophils send signals to alert immune cells (monocytes and other types of macrophages) to help destroy the bacteria or viruses that have entered the body (Soehnlein et al 2017). As shown in Figure 10, the percentage of neutrophils before treatment ranged from 19.40% to 22.2%. Based on the results obtained after the treatment, the percentage of neutrophils showed a decrease. This finding is consistent with the observations of Yuliana et al (2021), who reported a similar trend in the average percentage of neutrophils in the blood of pomfret (*Trachinotus blochii*). Their study showed that after administering feed containing *Sargassum* sp. extract, the neutrophil percentage tended to decrease. The percentage of neutrophils increased during the challenge test.

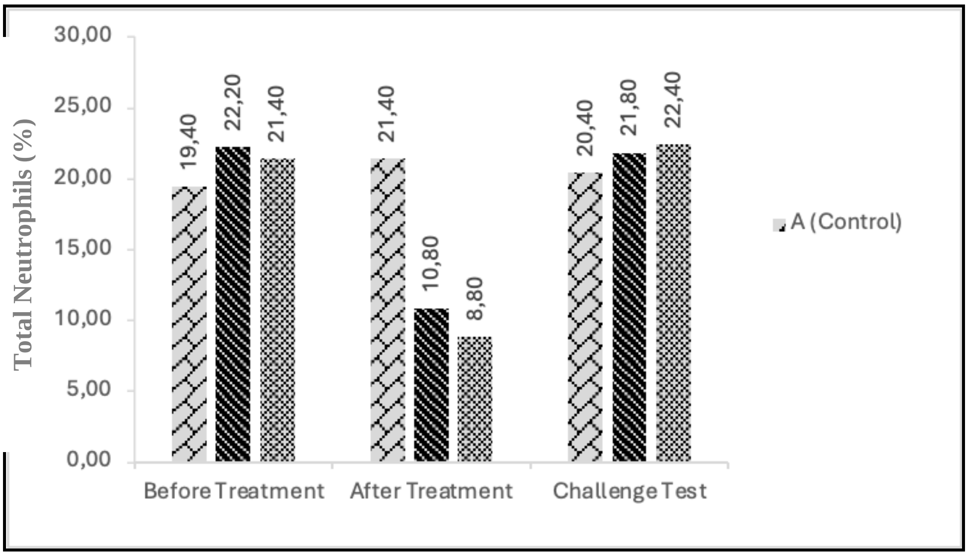

Figure 10. Percentage of neutrophils in cobia (*Rachycentron canadum*) fry during the research.

Clinical symptoms after infection

Body surface damage. In treatment B and C, the fish had showed no damage until 24 h, and presented hemorrhages in 32 h to 48 h of exposure (Table 4). Clinical symptoms in cobia fry began to appear in treatment A at the 12th hour post-infection, manifested by blackened bodies and the emergence of hemorrhagic red spots in the operculum area extending to the gill region (Figure 11d), while in treatments B and C, the clinical symptoms of blackened bodies and hemorrhage occurred at the 32nd hour. Clinical symptoms such as fin erosion (Figure 11c) began to appear in treatment A at the 24th hour post-infection with *V. alginolyticus* (Table 5). According to Rameshkumar et al (2017), cobia fry affected by vibriosis disease exhibit physical changes such as blackening of the body, followed by red spots on the fins, near the operculum and abdominal area, ulcerated lesions, swollen abdomen (dropsy), and protruding eyes (exophthalmia) (Figure 11). The onset of vibriosis disease symptoms usually occur between the 12th and 24th hours post-infection with the vibriosis agent (Nguyen et al 2017).

Table 4

Damage of body surfaces after 48 hours

Treatment	*Duration*					
	4 h	*6 h*	*12 h*	*24 h*	*32 h*	*48 h*
A	--	--	D	cd	cd	Cd
B	--	--	--	--	d	D
C	--	--	--	--	d	D

Note: b - ulcer; c - fin rot; d - hemorrhage.

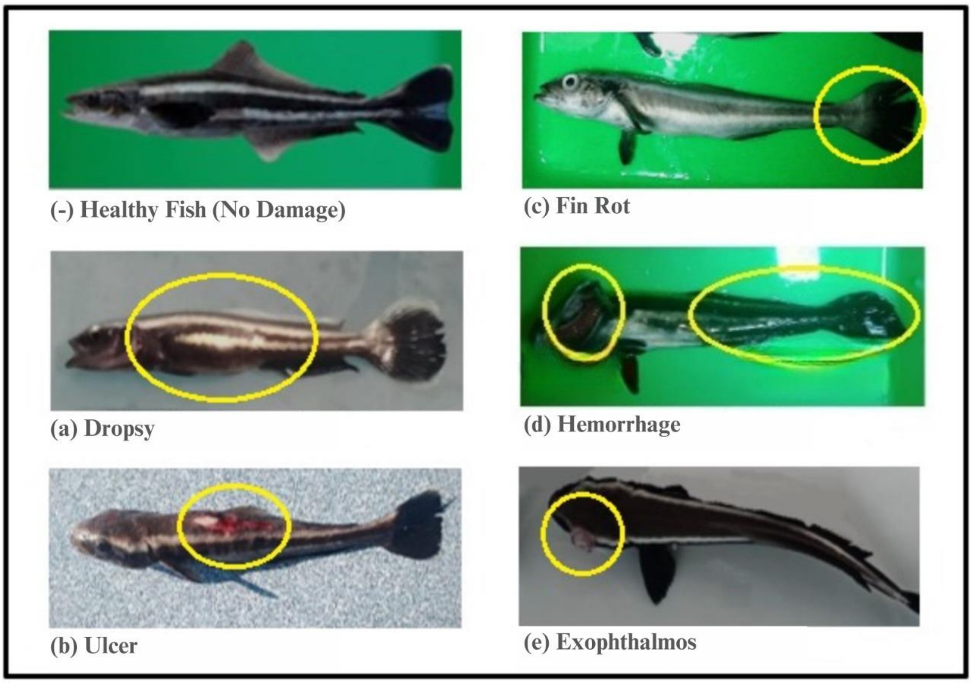

Figure 11. Clinical symptoms in cobia (*Rachycentron canadum*) fry.

Table 5

Damage of body surfaces after challenge test

Treatment	*Replication*	*Day*													
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
A	1	d	d	bcd	Abc	abc	bc	bcd	bcd	bc	b	--	--	--	--
	2	d	cd	bc	Abc	bce	bce	bcde	cde	ce	bce	be	e	E	E
	3	d	cd	bcd	Be	be	bce	bcde	ce	ce	e	e	--	--	--
	4	d	d	cd	Be	be	cde	ce	bce	bce	e	e	--	--	--
	5	--	cd	cd	bce	bce	cde	ce	ce	bce	ce	ce	c	--	--
B	1	--	--	d	C	c	bc	bce	bc	bc	b	b	--	--	--
	2	--	--	d	Bc	bc	bc	bc	bc	bc	b	--	--	--	--
	3	--	d	cd	Bc	bce	bc	bc	b	C	c	--	--	--	--
	4	--	d	c	Bc	bc	bc	bc	b	B	b	--	--	--	--
	5	--	d	cd	Bc	bc	bc	c	bc	Bc	b	--	--	--	--
C	1	--	d	d	C	c	c	c	bc	c	--	--	--	--	--
	2	--	--	d	C	c	c	bc	b	B	--	--	--	--	--
	3	--	--	d	C	c	c	b	b	B	b	--	--	--	--
	4	--	d	d	C	bc	bc	bc	b	B	b	--	--	--	--
	5	--	d	c	C	bc	bc	bc	b	B	b	--	--	--	--

Note: a - dropsy; b - ulcer; c - fin rot; d – hemorrhage; e - exophthalmos.

Survival rate. The SR did not have significant differences, because all treatments had an SR of 100%. However, after the 14-day challenge test, there were significant differences in the SR of cobia fry. The SR values after the challenge test showed that the best treatment was C, with a value of 90.7%. Treatment A had the lowest SR value of 66.7% (Figure 12). Treatments B and C had a significant influence on the SR of fry. Treatments B and C did not produce significant differences in SR. However, the SR in B and C are significantly different from that in treatment A, with treatment A producing the lowest SR.

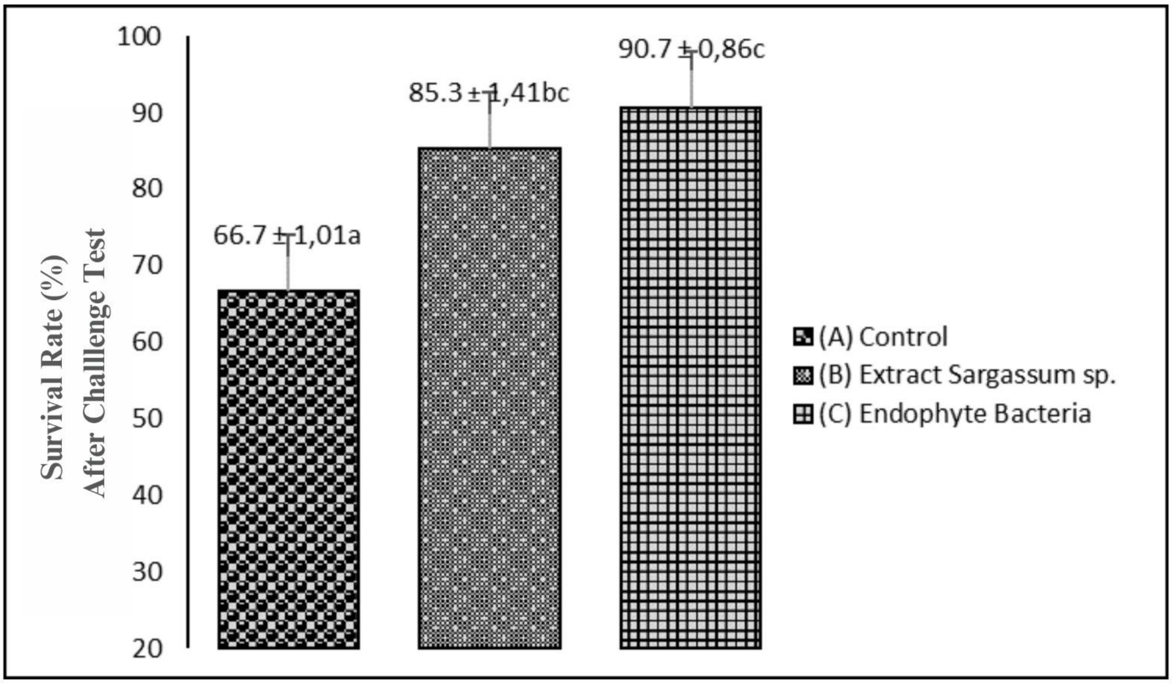

Figure 12. Survival rate of cobia (*Rachycentron canadum*) fry after the challenge test. Different letters above bars show significant differences ($p<0.05$).

Discussion. The addition of *Sargassum* extract to feed increases fish weight based on the chemical compounds contained in the algae extract. Sulfated polysaccharides (fucose, glucose, fucoidan, and mannose) and secondary metabolites (tannins, alkaloids, saponins) have antitumor, antiviral, antibacterial, antioxidant, and immunostimulant properties (Telles et al 2018). Sivagnanavelmurugan et al (2018) observed that adding *Sargassum wightii* extract containing 3% sulfated polysaccharides (fucoidan) increased feed intake and successfully enhanced SGR and average weight of *Penaeus monodon*.

In treatment C, the addition of endophytic bacteria isolated from *Sargassum* sp. produced secondary metabolites identical to its host. Although endophytic bacteria do not have the exact same major chemical compounds as *Sargassum*, they can act as active probiotics in enhancing immune defenses and facilitating digestive metabolism within the fish's body (Kenawy et al 2021).

Bioactive substances from *Sargassum* extract and endophytic bacterial isolates have effects on increasing fish feed intake. This is explained in the study of Grandiosa (2010), where bioactive substances such as alkaloids and phenols influence increased feed intake by enhancing palatability and metabolism rate. The feeding rate values of cobia fry in treatments B and C were 6.8 and 6.6%, respectively, with the control presenting a FR of 6%. The increased FR values indicate improved daily growth rates of cobia fry, indicating healthier fish and better digestion metabolism.

Differences in FCR are related to the digestibility of the feed administered. There is a slight difference in FCR values between treatments B and C, suggesting that the addition of endophytic bacterial isolates to the feed may be slightly superior to *Sargassum* extract due to the probiotic nature of the endophytic bacteria.

The increase in erythrocyte count in treatments B and C may be due to the effect of bioactive substances in *Sargassum* extract and endophytic bacteria. Pratiwy & Pratiwi

(2020) explained that *Sargassum* sp. extract contains several bioactive substances (alkaloids, triterpenoids, steroids, saponins, phenolics, and flavonoids) that function as immunostimulants, antibacterials, and antioxidants. The antioxidant activity mechanism in the body prevents an increase in free radicals and protects against early erythrocyte cell damage.

Endophytic bacteria have the ability to produce secondary metabolites (bioactive compounds) that function as immunostimulants and can also act as probiotics in the fish's digestive tract to enhance immunity. Probiotics work by improving nutrient absorption on the intestinal surface, stimulating the formation of intraepithelial leukocytes and goblet cells, and secreting antibacterial compounds (Pratiwy & Pratiwi 2020).

The percentage of lymphocyte count after the challenge test shows a decreasing trend in all treatments, indicating a decrease in leukocyte count due to infection with *V. alginolyticus*, causing inflammation and hyperaemia. Hyperaemia is the accumulation of blood in a specific tissue or organ due to inflammation, leading to capillary narrowing. Inflammation also triggers the migration of lymphocytes to infected tissue, where they recognize specific antigens to produce antibodies. Some lymphocytes perform phagocytosis on bacteria and then undergo lysis, signaling the body to produce differentiation of other leukocyte cells (monocytes and neutrophils) to help destroy pathogens entering the body (Pratiwy & Pratiwi 2020).

Conclusions. The incorporation of *Sargassum* extract and endophytic bacterial isolates into fish feed has shown promising effects on fish growth, feed intake, and immune response. Additionally, endophytic bacteria act as probiotics, aiding in nutrient absorption and boosting immunity within the fish's digestive system. These findings suggest that utilizing *Sargassum* algae extract and endophytic bacteria in fish feed formulations could be a valuable strategy for promoting sustainable aquaculture practices and improving fish health and productivity. Further research is warranted to optimize dosage levels and investigate long-term effects on fish growth and immune function.

Acknowledgements. The authors are grateful to the Rector of Padjadjaran University for the financial assistance provided through The Research Grant of Universitas Padjadjaran for Online Database Resources Grant (RDPD) to Fittrie M. Pratiwy funding scheme under contract No. 3626/UN6.3.1.1/PT.00/2021.

Conflict of Interest. The authors declare that there is no conflict of interest.

References

Ademola B. T., 2024 Enhancement of aquaculture performance of cobia, *Rachycentron canadum* (Linnaeus 1766): A review. International Journal of Aquaculture and Fisheries Sciences 10(1):1-8.

Ahmed E. F., Hassan H. M., Rateb M. E., Abdel-Wahab N., Sameer S., Ebel R., Taie H. A. A., Abdel-Hameed M. S., Hammouda O., 2016 A comparative biochemical study on two marine endophytes, Bacterium SRCnm and *Bacillus* sp. JS, isolated from red sea algae. Pakistan Journal of Pharmaceutical Sciences 29(1):17-26.

Amenyogbe E., Zhang J., Huang J., Chen G., 2022 The efficiency of indigenous isolates *Bacillus* sp. RCS1 and *Bacillus cereus* RCS3 on growth performance, blood biochemical indices and resistance against *Vibrio harveyi* in cobia fish (*Rachycentron canadum*) juveniles. Aquaculture Reports 25:101241.

Amlacher E., 1970 Textbook of fish disease. TFH Publ. Neptune, 302 p.

Besson M., Aubin J., Komen H., Poelman M., Quillet E., Vandeputte M., Van Arendonk J. A. M., de Boer I. J. M., 2016 Environmental impacts of genetic improvement of growth rate and feed conversion ratio in fish farming under rearing density and nitrogen output limitations. Journal of Cleaner Production 116:100-109.

Blaxhall P. C., Daisley K. W., 1973 Routine haematological methods for use with fish blood. Journal of Fish Biology 5(6):771-781.

Chart H., 2012 *Vibrio*, *mobiluncus*, gardnerella and *spirillum*: Cholera; vaginosis; rat bite fever. Medical microbiology (18th Edition). Churchill Livingstone, pp. 314-323.
Cui X. J., Zhou Q. C., Liang H. O., Yang J., Zhao L. M., 2010 Effects of dietary carbohydrate sources on the growth performance and hepatic carbohydrate metabolic enzyme activities of juvenile cobia (*Rachycentron canadum* Linnaeus.). Aquaculture Research 42(1):99-107.
Gaspersz, V., 1991 [Experiment design methods]. Cv Armico, 447 p. [In Indonesian].
Glamuzina B., Rimmer M. A., 2022 Grouper aquaculture world status and perspectives. In: Biology and ecology of groupers. 1st Edition. CRC Press, pp. 25.
Grandiosa R., 2010 [The effectiveness of using black cumin (*Nigella sativa*) filtrate solution with different concentrations on the in-vitro growth of *Aeromonas hydrophila* bacteria and its toxicity test on goldfish (*Cyprinus carpio*)]. Universitas Padjajaran, Jatinangor, 17 p. [In Indonesian].
Hasiani V. V., Ahmad I., Rijai L., 2015 [Isolation of endophytic fungi and production of antioxidant secondary metabolites from henna leaves (*Lawsonia inermis* L.)]. Jurnal Sains Dan Kesehatan 1(4):146-153. [In Indonesian].
Heinsbroek L. T. N., Kreuger J. G., 1992 Feeding and growth of glass eels, *Anguilla anguilla* L.: the effect of feeding stimulants on feed intake, energy metabolism and growth. Aquaculture Research 23(3):327-336.
Islam S. I., Mahfuj S., Baqar Z., Asadujjaman M., Islam M. J., Alsiwiehri N., Almehmadi M., Sanjida S., Ahammad F., 2024 Bacterial diseases of Asian sea bass (*Lates calcarifer*): A review for health management strategies and future aquaculture sustainability. Heliyon 10(9):e29793.
Kasanah N., Amelia W., Mukminin A., Triyanto, Isnansetyo A., 2019 Antibacterial activity of Indonesian red algae *Gracilaria edulis* against bacterial fish pathogens and characterization of active fractions. Natural Product Research 33(22):3303-3307.
Kenawy A., Abo-Zaid G., El-Gendi H., Hegazy G., Ho T., El Enshasy H., 2021 Probiotics applications in agriculture. In: Probiotics the natural microbiota in living organisms. CRC Press, pp. 251-291.Kusumaningrum I., 2007 [Effect of *Sargassum crassifolium* juice with different concentrations on the growth of soybean plants (*Glycine max* L. Merrill). Anatomi Fisiologi 15(2):17-23. [In Indonesian].
Lavilla-Pitogo C. L., Baticados M. C. L., Cruz-Lacierda E. R., de la Pena L. D., 1990 Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. Aquaculture 91(1-2):1-13.
Li M. H., Peterson B. C., Janes C. L., Robinson E. H., 2006 Comparison of diets containing various fish meal levels on growth performance, body composition, and insulin-like growth factor-I of juvenile channel catfish *Ictalurus punctatus* of different strains. Aquaculture 253(1-4):628-635.
Lugert V., Thaller G., Tetens J., Schulz C., Krieter J., 2016 A review on fish growth calculation: multiple functions in fish production and their specific application. Reviews in Aquaculture 8(1):30-42.
Mohamad N., Amal M. N. A., Yasin I. S. M., Saad M. Z., Nasruddin N. S., Al-saari N., Mino S., Sawabe T., 2020 Vibriosis in cultured marine fishes: a review. Aquaculture 512:734289.
Mohammed M. H., Abogabal I. I., Ibrahim A. H., Maaty M. M., Farahat A. Z., El-Regal M. A. A., 2021 Molecular characterization of *Vibrio* spp. isolated from naturally infected larvae of the delicate round herring *Spratelloides delicatulus* (Pisces: Clupeidae) in the Red Sea. Journal of King Abdulaziz University: Marine Sciences 30(1):17-28.
Nguyen H. T., Nguyen T. T. T., Wang Y. T., Wang P. C., Chen S. C., 2017 Effectiveness of formalin-killed vaccines containing CpG oligodeoxynucleotide 1668 adjuvants against *Vibrio harveyi* in orange-spotted grouper. Fish & Shellfish Immunology 68:124-131.
Permatasari R., Rozirwan R., Saputra, S., 2019 [The effect of giving different immunostimulant doses in artificial feed on the growth rate and survival of cobia fish (*Rachycentron canadum*)]. Sriwijaya University, 39 p. [In Indonesian].
Pratiwy F. M., Pratiwi D. Y., 2020 The effects of bioactive compound (antioxidant) from *Sargassum* extract on the erythrocytes and differential leucocytes of catfish (*Clarias*

sp.). International Journal of Fisheries and Aquatic Studies 8(6):123-126.
Putra E. M., Mahasri G., Sari L. A., 2018 [Ectoparasite infestation in tilapia (*Oreochromis niloticus*) raised using aquaponics and without aquaponics systems]. Journal of Aquaculture and Fish Health 7(1):42-49. [In Indonesian].
Rafsyanzani M. M., Hidayatullah D., 2016 [Performance of probiotic *Bacillus* sp. in the catfish nursery *Clarias* sp. infected with *Aeromonas hydrophila*]. Jurnal Akuakultur Indonesia 15(2):162-170. [In Indonesian].
Rajan P. R., Lopez C., Lin J. H., Yang H., 2001 *Vibrio alginolyticus* infection in cobia (*Rachycentron canadum*) cultured in Taiwan. Bulletin of the European Association of Fish Pathologists 21(6):228-234.
Rameshkumar P., Kalidas C., Tamilmani G., Sakthivel M., Nazar A. K. A., Maharshi V. A., Rao S. K. R., Gopakumar G., 2014 Microbiological and histopathological investigations of *Vibrio alginolyticus* infection in cobia *Rachycentron canadum* (Linnaeus, 1766) cultured in sea cage. Indian Journal of Fisheries 61(1):124-127.
Rameshkumar P., Nazar A. K. A., Pradeep M. A., Kalidas C., Jayakumar R., Tamilmani G., Sakthivel M., Samal A. K., Sirajudeen S., Venkatesan V., Nazeera B. M., 2017 Isolation and characterization of pathogenic *Vibrio alginolyticus* from sea cage cultured cobia (*Rachycentron canadum* (Linnaeus 1766)) in India. Letters in Applied Microbiology 65(5):423-430.
Riwanti P., Andayani R., Trinanda L., 2021 [Antibacterial activity test of *Sargassum polycystum* against *Staphylococcus aureus* bacteria]. Journal of Pharmacy and Science 6(1):19-23. [In Indonesian].
Rojo-Cebreros A. H., Ibarra-Castro L., Martínez-Brown J. M., 2018 Immunostimulation and trained immunity in marine fish larvae. Fish & Shellfish Immunology 80:15-21.
Sem N. A. D. K., Gani S. A., Chong C. M., Natrah I., Shamsi S., 2023 Management and mitigation of vibriosis in aquaculture: Nanoparticles as promising alternatives. International Journal of Molecular Sciences 24(16):12542.
Sivagnanavelmurugan M., Radhakrishnan S., Palavesam A., Arul V., Immanuel G., 2018 Characterization of alginic acid extracted from *Sargassum wightii* and determination of its anti-viral activity on shrimp *Penaeus monodon* post larvae against white spot syndrome virus. International Journal of Current Research in Life Sciences 7(4):1863-1872.
Soehnlein O., Steffens S., Hidalgo A., Weber C., 2017 Neutrophils as protagonists and targets in chronic inflammation. Nature Reviews Immunology 17:248-261.
Sujatha R., Siva D., Nawas P., 2019 Screening of phytochemical profile and antibacterial activity of various solvent extracts of marine algae *Sargassum swartzii*. World Scientific News 115:27-40.
Sukmawaty E., Masri M., Putri S. U., Nurzakiyah N., 2016 [Antibacterial activity of extracts and endophytic bacteria of the macro alga *Caulerpa racemosa* L. from Puntondo waters against *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* (MRSA)]. Prosiding Seminar Nasional from Basic Science to Comprehensive Education, Universitas Islam Negeri Alauddin Makassar, pp. 174-179. [In Indonesian].
Telles C. B. S., Mendes-Aguiar C., Fidelis G. P., Frasson A. P., Pereira W. O., Scortecci K. C., Camara R. B. G., Nobre L. T. D. B., Costa L. S., Tasca T., Rocha H. A. O., 2018 Immunomodulatory effects and antimicrobial activity of heterofucans from *Sargassum filipendula*. Journal of Applied Phycology 30(1):569-578.
Yuliana A., Wulandari R., Zahra A. 2021 [Giving *Sargassum* sp. extract through commercial feed on hematocrit values and differential leukocytes in pomfret *Trachinotus blochii*]. Intek Akuakulur 5(2):36-49. [In Indonesian].
Zhao Z., Zhang C., Jia Y. J., Qiu D. K., Lin Q., Li N. Q., Huang Z. B., Fu X. Z., Wang G. X., Zhu B., 2019 Immersion vaccination of Mandarin fish *Siniperca chuatsi* against infectious spleen and kidney necrosis virus with a SWCNTs-based subunit vaccine. Fish & Shellfish Immunology 92:133-140.
*** Badan Standardisasi Nasional (Indonesian Standard Organization), 2004 [SNI 06-6989.11-2004: Water and wastewater-Part 11: How to test the degree of acidity (pH) using a pH meter]. [In Indonesian].

*** BBPBL, 2020 [Cobia fish cultivation (*Rachycentron canadum*) BBPBL Lampung]. Balai Besar Perikanan Budidaya Laut Lampung TA. [In Indonesian].

Received: 24 April 2024. Accepted: 29 May 2024. Published online: 13 August 2024.
Authors:
Fittrie Meyllianawaty Pratiwy, Department of Fisheries, Faculty of Fishery and Marine Science, Universitas Padjadjaran, Jatinangor, Sumedang, 45363 West Java, Indonesia, e-mail: fittrie.pratiwy@unpad.ac.id
Muhammad Irfan Sumantri, Study Program of Fisheries Science, Faculty of Fishery and Marine Science, Universitas Padjadjaran, Jatinangor, Sumedang, 45363 West Java, Indonesia e-mail: keealf@hotmail.com
Suryadi Saputra, Research Center of Fisheries, National Research and Innovation Agency, 10340 Jakarta, Indonesia, e-mail: muh.irfan.sumantri@gmail.com
Rosidah, Department of Fisheries and Study Program of Fisheries Science, Faculty of Fishery and Marine Science, Universitas Padjadjaran, Jatinangor, Sumedang, 45363 West Java, Indonesia, e-mail: rosidah@unpad.ac.id
Walim Lili, Department of Fisheries and Study Program of Fisheries Science, Faculty of Fishery and Marine Science, Universitas Padjadjaran, Jatinangor, Sumedang, 45363 West Java, Indonesia e-mail: walim.lili@unpad.ac.id
Iskandar, Department of Fisheries and Study Program of Fisheries Science, Faculty of Fishery and Marine Science, Universitas Padjadjaran, Jatinangor, Sumedang, 45363 West Java, Indonesia e-mail: iskandar@unpad.ac.id
Thallita Nasywa Faddilah, Study Program of Marine Conservation, Faculty of Fishery and Marine Science, Universitas Padjadjaran, Jatinangor, Sumedang 45363, West Java, Indonesia, e-mail: thallita19001@mail.unpad.ac.id
Rafidah Othman, Borneo Marine Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, 88400 Sabah, Malaysia, e-mail: rafidaho@ums.edu.my

How to cite this article:
Pratiwy F. M., Sumantri M. I., Saputra S., Rosidah, Lili W., Iskandar, Faddilah T. N., Othman R., 2024 Exploring the potential of endophytic bacteria isolated from *Sargassum* sp.: Enhancing growth and non-specific immune response of cobia *Rachycentron canadum* (Linnaeus, 1766). AACL Bioflux 17(4):1585-1601.