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HEMATOLOGICAL RESPONSE OF *Pangasianodon hypophthalmus* FED *Cosmos caudatus* ENRICHED FEED AND REARED IN SALINE MEDIA

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ABSTRACT

Raising striped catfish (*Pangasianodon hypophthalmus*) in dark conditions with low salinity (5 ppt) can support growth but may also cause stress and affect blood health. Adding *Cosmos caudatus* leaves, which contain natural bioactive compounds, in fish feed could offer a natural way to help boost the immune system. This study aimed to understand the hematological response of fish toward the *C.caudatus* enriched feed provision. The fish were reared for 60 days in 5 ppt salinity water and under a controlled photoperiod. There were four treatments: control (no *C.caudatus*) T1, T2 and T3 (10 g, 20 g, and 30 g of *C.caudatus* leave powder in 1 kg of feed). Parameters observed were red and white blood cell number, hemoglobin, hematocrit and glucose concentration, phagocytic activity, leukocrit, and types of white blood cells. Results showed that the T1 group significantly improved the ability of red blood cells, hemoglobin, hematocrit, and phagocytosis. Survival rates were high and showed no significant differences between treatments, suggesting that *C.caudatus* up to 30 g/kg is safe. Based on these findings, a dose of 10 g/kg can be recommended as an effective natural feed additive to help improve the health and immune system of *P.hypopthalmus* cultured in controlled rearing conditions.

Keywords: Striped Catfish, Natural Herbs, Feed Additive, Fish Blood, Salinity

1. INTRODUCTION

Striped catfish (*Pangasianodon hypophthalmus*) is a leading commodity in freshwater aquaculture with fast growth and high economic value. As a nocturnal fish, rearing in dark conditions can increase feed consumption and growth[1,2]. However, this condition also reduces water quality and increases the risk of infection due to the accumulation of organic matter[3].

Salinity is one of the factors that can be modified to suppress pathogens. The addition of salinity is known to increase the mucus of the fish body and its resistance to infection[4]. Catfish can survive up to 15 ppt, but the optimal salinity for growth is around 5 ppt[5]. However, increased salinity also has the potential to cause osmoregulatory stress that affects hematological parameters such as erythrocytes, hemoglobin, and glucose[6-9].

Feeding enriched kenikir (*Cosmos caudatus*) leaves is a promising natural strategy because of their flavonoids, saponins, and polyphenols, which are antioxidant, antimicrobial, and immunostimulant[10]. These compounds are thought to stabilize the hematological response of fish in challenging environmental conditions. However, studies on the effect of kenikir leaf-based feed on the hematology of Striped catfish in dark media with salinity are still limited. This study aims to evaluate the effect of feeding enriched with kenikir leaves on the hematological profile of Striped catfish reared under these conditions.

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2. RESEARCH METHOD

Time and Place

The research was conducted for 60 days from January to March 2025 at the Aquatic Biology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau.

Method

The study used a one-factor Completely Randomized Design (CRD) with four treatments and three replicates, namely: P0 (feed without kenikir leaves - control); P1 (feed + 10 g/kg kenikir leaves); P2 (feed + 20 g/kg kenikir leaves); P3 (feed + 30 g/kg kenikir leaves).

Procedures

Preparation of Maintenance Media and Feed

Striped catfish with an average size of 7 cm were reared in 100 L buckets containing 90 L of 5 ppt salinity water. Each bucket was covered with a tarp to create dark conditions (photoperiod) and equipped with a circulation and filtration system using palm fiber, ginger coral, and dacron. The feed was commercial pellets enriched with kenikir leaf meal according to the treatment dose.

Fish Maintenance

After 7 days of adaptation, fish were reared in a bucket containing 90 L of water with a stocking density of 30 fish/container (1 fish/3L). Initial data on fish length and weight were recorded before maintenance. Feed was given three times a day (09:00, 12:00, and 17:00 WIB) using the ad satiation method.

Research Parameters

Hematological parameters were observed 3 times on the 0th, 30th, and 60th days. Fish blood sampling was carried out by taking blood samples from catfish, with as much as 1 sample per unit of research container taken randomly.

Survival Rate

Survival was calculated at the end of the observation using the formula from Effendie[11].

$$SR = \frac{Nt}{No} \times 100\%$$

Description:
SR = Survival rate (%)
No = Initial number of fish (fish)
Nt = Final fish count (fish)

Total Erythrocytes

The number of erythrocytes was calculated using Klonz's method in Wandi[12]. A total of 0.5 mL of blood was taken using a capillary pipette containing a stirring stone, then diluted with Hayem's solution to a volume of 101 mL. The mixture was homogenized by shaking in a figure-eight shape, and then the first two drops were discarded to remove air bubbles. One drop of solution was dropped into the haemocytometer's counting chamber, which had been covered with a cover glass, then observed under a microscope with a magnification of 400× in five fields of view. The number of erythrocytes was calculated using the Blaxhall & Daisley formula in Allifuddin[13].

Total Erythrocytes= $\sum n \times 10^4$ cell/mm^3

Description:
$\sum n$: Total number of cells in 5 field of view
10^4 : Dilution factor

Total Leukocytes

The leukocyte count was calculated based on Klonz's method in Wandi[12]. A total of 0.5 mL of blood was taken using a capillary pipette containing a white stirring stone, then diluted with Turk's solution to a volume of 11 mL. The mixture was homogenized by shaking in a figure-eight shape, and then the initial two drops were discarded to remove air bubbles. One drop of solution was dripped into the counting chamber of the haemocytometer that had been covered with a cover glass, then observed under a microscope with a magnification of 400× at four fields of view in a large box. Leukocyte counts were

calculated using the Blaxhall and Daisley formula in Allifuddin[13].

Total leukocytes =∑ n x 50 cell/mm^3

Description:

∑n : Total number of cells in 4 field of view

50 : Dilution factor

Hemoglobin

Hemoglobin levels were measured by the Sahli method[14]. Blood as much as 0.02 mL was put into a Hb-meter tube containing 0.1 N HCl to a volume of 10 mL, stirred and allowed to stand for 3-5 minutes. Distilled water was added gradually until the color of the solution matched the standard, and then the hemoglobin level was read on the g/100 mL scale.

Glucose

Measurement of glucose levels was carried out using a glucometer. First, the tools and materials to be used, glucose strips are inserted into the glucometer, then blood is dripped on the sample zone slowly until a click is heard, after which the results will appear on the screen within 5 seconds; the results that appear are then recorded, and the strip is removed from the tool. The results are read in mg/dl. The screen will automatically show the code and signs of blood droplets.

Hematocrit and Leukocrit

Hematocrit and leukocrit were calculated using the formula according to Anderson & Siwicki in Allifuddin[13].

$$\text{Hematokrit \& Leukokrit} = \frac{\text{Blood cell deposits in capillary tubes}}{\text{Total length}} \text{ x } 100\%$$

Leukocyte Differentiation

Calculation of leukocyte types based on the Blaxhal and Daisley method in Allifuddin[13], namely fish blood is taken and made a blood screw preparation on an object glass, then dried, then fixed with 95% methanol for 5 minutes, rinse with distilled water and then dry. Next, stain with Giemsa for 15 minutes, wash with running water, dry and observe under a microscope, counting the types of leukocytes, namely, lymphocytes, platelets, neutrophils and monocytes, until the number is 100 cells.

Phagocytosis Index

Phagocytosis activity was measured by the modified method of Witeska et al.9 Blood swab preparations were stained with a combination of Giemsa and Carbol Fuchsin, then observed under a 1000x microscope until 100 cells were counted. Phagocytic activity was calculated as the percentage of phagocytic cells containing bacteria.

$$\text{Activities of phagocytic} = \frac{\text{Amount of phagocytic}}{\text{100 Leukocytes cell}}$$

Water Quality

Observed water quality parameters such as temperature and pH were measured using a thermometer and pH meter according to SNI[15]. Dissolved oxygen was measured with a DO meter. Salinity was measured using a refractometer. Ammonia levels were analyzed by a spectrophotometric method based on SNI 06-6989.30-2005.

Data Analysis

Data were statistically analyzed using ANOVA with SNL further test at 95% confidence level ($p<0.05$) to see the treatment effect between groups.

3. RESULT AND DISCUSSION

Survival Rate

After 60 days of rearing, the administration of various doses of kenikir leaves did not significantly affect the survival rate of Striped catfish. ANOVA analysis showed a significance value of 0.188 ($P > 0.05$) and an F count of 2.031, indicating that the difference in survival between treatments was not statistically significant. These results indicate that using miscellaneous leaves up to a dose of 20 g/kg is safe and does not cause toxic effects or physiological stress to the fish, even when applied under environmental conditions of salinity and dark lighting. Thus, using

kenikir leaves up to a dose of 20 g/kg is still within safe limits for the life of Striped catfish. The survival rate for each treatment during the study can be seen in Table 1.

Table 1. Survival during 60 days of rearing

Repeat	Survival rate (%)			
	P0	P_1	P_2	P_3
1	93,33	96,67	93,33	93,33
2	86,67	93,33	100,00	93,33
3	93,33	100,00	96,67	96,67
Average	91,11±3,84[a]	96,66±3,33[a]	96,66±3,33[a]	94,44±1,93[a]

Total Erythrocytes

Observations showed that fish fed with food enriched with kenikir leaves had more erythrocytes than the control. On day 30, P1 (10 g/kg) produced the highest erythrocytes (1.36±0.02[b]), significantly different from the control (1.19±0.01[a]), while P2 and P3 increased but not significantly. After 60 days, the increase in erythrocytes in P1 (1.41±0.01[d]), P2 (1.37±0.01[c]), and P3 (1.33±0.01[b]) was significant compared to the control (1.22±0.01[a]). All values are within the normal physiological range between 1.05-3.00 million cells/mm3[16]. This increase is due to the bioactive compounds of kenikir leaves, such as flavonoids and tannins that act as immunostimulants and antioxidants. Conversely, low erythrocytes in controls may indicate stress or decreased endurance[17]. The total number of erythrocytes in each treatment during the study can be seen in Table 2.

Table 2. Total erythrocytes during 60 days of maintenance

Repeat	Total erythrocytes ($x10^6$ cells/ mm^3)	
	Day 30	Day 60
P0	1.19±0.01[a]	1.22±0.01[a]
P1	1.36±0.02[b]	1.41±0.01[d]
P2	1.31±0.01[ab]	1.37±0.01[c]
P3	1.28±0.01[ab]	1.33±0.01[b]

Hemoglobin

The results showed that the hemoglobin level of striped catfish fed with food enriched with kenikir leaves tended to be higher than the control on day 30 and day 60. On day 30, treatment P1 recorded the highest level (7.33±0.11[b]) and significantly differed from the control (6.60 ± 0.20[a]). Treatments P2 (7.06 ± 0.11[b]) and P3 (7.00±0.20[b]) also showed a significant increase. The same pattern continued at day 60, where P1 still recorded the highest hemoglobin level (7.53±0.11[b]), while the control remained the lowest (6.86±0.23[a]). This increase suggests that bioactive compounds in kenikir leaves, such as flavonoids and tannins, play a role in the stimulation of hematopoietic organs and hemoglobin protein repair[18,19].

High hemoglobin levels reflect good physiological and metabolic status, supporting growth and resistance to environmental stress. Conversely, low levels may indicate impairment due to infection or stress[20-21]. An increase in hemoglobin aligns with an increase in erythrocytes, as they are physiologically interrelated[22]. Hemoglobin levels in each treatment during the study can be seen in Table 3.

Table 3. Hemoglobin during 60 days of maintenance

Repeat	Hemoglobin	
	Day 30	Day 60
P0	6.60±0.20[a]	6.86±0.23[a]
P1	7.33±0.11[b]	7.53±0.11[b]
P2	7.06±0.11[b]	7.40±0.20[b]
P3	7.00±0.20[b]	7.13±0.11[ab]

Hematocrit

Along with the increase in erythrocytes and hemoglobin levels in the fish group treated with kenikir leaves compared to the control fish group (P0), the hematocrit value also showed a similar

picture. The highest hematocrit value was found in the P1 treatment (31.00 ± 1.00% on day 30 and 34.33 ± 0.57% on day 60), significantly higher than the control. This indicates that the dose of 10 g/kg feed of kenikir leaves increased the number of red blood cells, contributing to increased oxygen-carrying capacity in the blood. Hematocrit in each treatment during the study can be seen in Table 4.

Table 4. Hematocrit during 60 days of maintenance

Repeat	Hematocrit (%)	
	Day 30	Day 60
P0	28.00±1.00^{a}	31.00±1.00^{a}
P1	31.00±1.00^{b}	34.33±0.57^{b}
P2	28.66±0.57^{ab}	33.00±1.00^{b}
P3	28.00±1.00^{a}	31.33±0.57^{a}

The increase in hematocrit values observed in the P1 and P2 treatments indicates an increase in the number of erythrocytes, which reflects the better physiological and metabolic conditions of the fish. A decreased hematocrit can occur in fish exposed to pathogenic bacteria without immunostimulants[18]. Thus, the relatively stable increase in P1 and P2 means that the fish in the treatment with kenikir showed an improvement in blood quality due to increased nutrition from the feed.

Total Leukocytes

The total number of leukocytes in each treatment during the study can be seen in Table 5.

Table 5. Total leukocytes during 60 days of maintenance

Repeat	Total Leukocytes (x10^{4}cells/mm^{3})	
	Day 30	Day 60
P0	7.62±0.01^{a}	7.83±0.01^{a}
P1	7.82±0.01^{d}	8.06±0.04^{d}
P2	7.78±0.02^{c}	7.96±0.02^{b}
P3	7.70±0.02^{b}	7.91±0.02^{b}

The results showed that the addition of kenikir leaves to the feed provided significant differences in the number of leukocytes in each treatment, both on day 30 and day 60. On day 30, treatment P1 showed the highest leukocyte count (7.82±0.01^{d}), followed by P2 (7.78±0.02^{c}), P3 (7.70±0.02^{b}), and control (7.62±0.01^{a}). On the 60th day, the leukocyte value also increased in the same order, and P1 remained the highest treatment (8.06 ± 0.04^{d}). This indicates that the administration of kenikir leaves can increase the immune response of fish gradually and sustainably.

All leukocyte values were within the normal physiological range of 20,000-150,000 cells/mm^{3} or 2.0-15.0 x10^{4} cells/mm^{3}[23], indicating that all fish were healthy. The increase in leukocyte count, especially at a dose of 10 g/kg (P1), indicates the stimulation of the immune system. This is thought to be influenced by the content of bioactive compounds in kenikir leaves, such as flavonoids and tannins, that can stimulate the formation of lymphocytes and monocytes, two critical leukocytes in the immune response[24].

Leukocrit

Leukocrit data on each treatment during the study can be seen in Table 6.

Table 6. Leukocrit during 60 days of maintenance

Repeat	Leukocrit (%)	
	Day 30	Day 60
P0	2.66±0.57	3.66±0.57
P1	2.66±0.57	3.33±0.57
P2	2.66±0.57	2.66±0.57
P3	3.00±1.00	3.66±0.57

Leukocrit describes the percentage volume of leukocytes in the blood, which plays a vital role in the immune system of fish, so an increase in the number of leukocytes will increase the level of leukocrit. Increased leukocyte count is one of the parameters of the immune response[25]. If a specific pathogen infects fish, there will be an increase or decrease in the total number of leukocytes[8].

Observations showed that leukocrit values were relatively stable in all treatments but increased slightly on day 60, especially in control (P0) and P3 treatments (3.66±0.57%). This increase may be due to immunological responses to changing environmental conditions or the presence of pathogenic agents. Leukocrit values that tend to be stable in the range of 2.66 to 3.66% indicate that, in general, the fish can still maintain an effective immune response.

Overall, leukocyte values were still within the normal physiological range of 20,000 to 150,000 cells/mm³ or 2.0 - 15.0 x10⁴ cells/mm³[23], indicating that all treatments were still within healthy limits but with different levels of immune response. The increase in leukocyte count in the treatment with kenikir leaves, especially the 10 g/kg dose (P1), indicates the stimulation of the fish immune system. The bioactive content of kenikir leaves, such as flavonoids and tannins, are thought to stimulate the production of lymphocytes and monocytes, two types of leukocytes that are important in dealing with infection[24]. Nutritional factors, especially protein in feed, also contribute to the formation of leukocytes [26,] and it is likely that bioactive in kenikir also increases the efficiency of protein metabolism.

Glucose

Blood glucose is an important physiological parameter that reflects fish's metabolic status and stress level. On day 30, treatment P2 showed the highest glucose level (104.00±5.56 mg/dL), while P1 was the lowest (85.00±20.66 mg/dL). Glucose values tended to stabilize on day 60, especially in P1 (91.33±5.13 mg/dL) and P3 (89.33±11.93 mg/dL), which were within the normal range (40-90 mg/dL)[27]. Meanwhile, the glucose value in the control (P0) decreased to 83.33±22.50 mg/dL. The P2 treatment still showed relatively high levels (93.66±17.03 mg/dL). Glucose in each treatment during the study can be seen in Table 7.

Table 7. Glucose during 60 Days of Maintenance

Repeat	Glucose (mg/dL)	
	Day 30	Day 60
P0	99.66±7.50	83.33±22.50
P1	85.00±20.66	91.33±5.13
P2	104.00±5.56	93.66±17.03
P3	94.33±15.94	89.33±11.93

This may be caused by bioactive compounds in kenikir leaves, such as flavonoids that act as antioxidants[28]. Conversely, high glucose levels in the P2 treatment may indicate stress due to the dose being too high. According to Djauhari et al.[29], higher blood glucose levels indicate higher fish stress levels. Fluctuations in glucose levels in this study are still within a reasonable physiological range, but the stability of glucose levels in P1 indicates that the dose is the most optimal in maintaining metabolism and reducing stress in striped catfish.

Leukocyte Differentiation

The white blood cells in the treated fish were lymphocytes, monocytes, neutrophils, and platelets. Leukocyte differentiation data in each treatment during the study can be seen in Table 8.

The results showed that lymphocytes were the dominant cells with a proportion of 72.33 -75.66%, the highest in treatment P1 (75.66% day 60), indicating that a dose of 10 g/kg of kenikir leaves was able to optimize the adaptive immune response of fish. This proportion is still within the normal range (58.83-74.87%), according to Lukistyowati[30]. Monocytes (8.66-10%) and neutrophils (7-8.66%) were stable in all treatments, indicating the absence of severe infection and phagocytic activity that remained normal[31]. Platelets were also stable, indicating no physiological disturbances. Overall, a 10 g/kg dose of kenikir leaves increased lymphocytes without disturbing the immunological balance of the fish.

Table 8. Leukocyte differentiation during 60 days of maintenance

Parameters	Leukocyte Differentiation			
	P0	P1	P2	P3
Day 30				
Lymphocytes	72.33±0.57	74.33±0.57	72.66±0.57	72.33±0.57
Monocytes	9.33±0.57	8.66±1.52	8.66±1.52	10.00±2.00
Neutrophils	8.66±0.57	7.66±0.57	8.33±0.57	8.33±0.57
Platelets	9.66±1.15	9.33±1.52	10.33±0.57	9.33±1.52
Day 60				
Lymphocytes	73.00±1.00	75.66±0.57	73.00±1.00	73.00±1.00
Monocytes	10.00±1.00	9.33±1.52	10.00±3.00	10.00±2.00
Neutrophils	7.33±0.57	7.00±1.00	7.33±0.57	7.66±0.57
Platelets	9.66±0.57	8.00±1.00	9.66±2.08	9.33±1.15

Phagocytosis Index

Phagocytosis activity is one of the crucial indicators of non-specific immune responses in fish. This activity reflects how effectively the fish immune system protects the body from pathogen attacks[32]. Phagocytosis data on each treatment can be seen in Table 9.

Table 9. Phagocytosis during 60 days of maintenance

Repeat	Phagocytosis	
	Day 30	Day 60
K	17.00±1.00^{a}	21.00±1.00^{a}
P1	21.66±0.57^{b}	25.00±1.00^{b}
P2	20.00±1.00^{ab}	22.00±1.00^{a}
P3	19.67±0.57^{ab}	21.33±0.57^{a}

The provision of kenikir leaves in feed was shown to increase Striped's phagocytosis activity. Treatment P1 (10 g/kg) showed the highest value, which was 21.66 ± 0.57% on day 30 and 25.00 ± 1.00% on day 60, significantly different from the control (17.00 ± 1.00% and 21.00 ± 1.00%). The increase in phagocytosis activity is thought to be triggered by bioactive compounds in kenikir leaves, such as flavonoids and tannins, which play a role in activating macrophages through macrophage activating factor (MAF), which trigger the expression of immune genes[33].

P2 (20 g/kg) treatment resulted in 22.00 ± 1.00% activity on day 60, while P3 (30 g/kg) decreased to 21.33 ± 0.57%, indicating a possible decrease in effectiveness due to over-dosing. P1 treatment provided the most optimal effect in stimulating phagocytosis activity, while P3 decreased the activity. This shows the importance of determining the optimal dose using natural ingredients such as kenikir leaves to support the fish's immune system.

Water Quality

Water quality is a very important factor in supporting the growth and survival of fish. Water quality measured during the study were pH, temperature, dissolved oxygen and ammonia (NH_3). The results of water quality measurements can be seen in Table 10.

Table 10. Water quality during 60 days of rearing

Parameters	Day 0	Day 30	Day 60
Temperature^{0}C	26-27	27-28	27-29
pH	7,2-7,5	7,2-7,7	7,2-7,6
DO (ppm)	6,4-7,6	3,7-5,4	3,8-5,2
Ammonia (Mg/L)	0,13	0,41-0,73	0,49-1,68
Nitrate (Mg/L)	0,2	6,06-23,11	2,69-22,9
Phosphate (Mg/L)	0,6	14,94-16,73	16,05-19,90

During the rearing period, the water temperature ranged from 26°C to 29°C. This range is still considered optimal for the growth of striped catfish because temperature affects metabolic rate and oxygen solubility. A stable temperature also supports appetite and growth. The pH value ranges from 7.2-7.7, classified as neutral to slightly alkaline, which is still suitable for freshwater fish farming. pH affects the biological activity of fish and the availability of chemical elements in water[11], and these values do not cause physiological stress in fish.

Dissolved oxygen (DO) levels decreased from 6.4-7.6 mg/L at the beginning to 3.8-5.2 mg/L on day 60. This decrease was thought to be due to increased biomass and decomposition of organic matter. Although still within the tolerance range, DO close to the threshold (≥4 mg/L) can potentially trigger stress and reduce metabolic efficiency[34].

Ammonia levels increased during rearing, reaching 1.68 mg/L on day 60. Ammonia results from the decomposition of leftover feed and faeces, which in free form (NH_3) is toxic to fish. Ammonia content is strongly influenced by temperature and pH[35] and can cause gill irritation and reduce growth rate.

Nitrate and phosphate concentrations also increase with organic matter accumulation and fish excretion. Although not directly toxic, their accumulation can trigger eutrophication and water quality degradation through algae population explosion.

In general, fluctuations in water quality, such as temperature, pH, DO, ammonia, nitrate and phosphate, showed similar patterns in all treatments. No significant differences were found between treatments related to the provision of kenikir leaves, so it can be concluded that the supplementation of kenikir leaves did not significantly affect water quality. Although some parameters showed decreased quality at the end of rearing, all values were within tolerance limits for striped catfish farming. Therefore, water quality management, such as water changes and feed management, must be considered to maintain fish health and performance.

4. CONCLUSION

Striped catfish's hematological health and immune system cultured under challenging environmental conditions, namely 5 ppt salinity and dark lighting. Among the various doses tested, 10 g/kg feed (treatment P1) of kenikir leaves produced the most optimal response, characterized by significant increases in the parameters of erythrocytes, hemoglobin, hematocrit, leukocytes, and phagocytosis activity. The P1 treatment produced the highest erythrocyte count of 2.55 $x10^6$ cells/mm^3, hematocrit value of 31%, and leukocyte count of 7.84 $x10^4$ cells/mm^3, indicating good physiological and immune status of the fish. In addition, the proportion of lymphocytes as the dominant immune cell also increased, reflecting the strengthening of the adaptive immune response.

Thus, kenikir leaves have great potential as a cheap, easily obtained, and environmentally friendly natural feed additive to support intensive and semi-intensive striped catfish farming systems, especially in cultivation conditions with 5 ppt salinity and dark media. A dose of 10 g/kg feed is recommended to improve hematological performance and fish endurance without causing toxic effects or stress. Using natural additives such as this can be a sustainable strategy in developing modern aquaculture that emphasizes production efficiency and fish welfare.

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