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Research Article

Effect of Dietary *Canarium indicum* L. on the Growth, Health, and Resistance of Asian Seabass Challenged with *Vibrio alginolyticus*

Taufiq Abdullah, Dinamella Wahjuningrum*, and Widanarni Widanarni

Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Bogor, West Java, 16680. Indonesia

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*) Corresponding author:
E-mail: dinamellawa@apps.ipb.ac.id

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Abstract

Canarium indicum offers the potential of usage in aquaculture. This study aimed to evaluate the utilization of *C. indicum* on growth performance, health, and resistance of Asian seabass *Lates calcalifer* challenged with *Vibrio alginolyticus*. The study employed a completely randomized design with seven treatments, namely positive control (K+), negative control (K-), vitamin control (KVit), antibiotic control (KAnt), doses of 5 g kg^{-1} (D05), 10 g kg^{-1} (D10), and 20 g kg^{-1} *C. indicum* (D20). The test feed was prepared with a coating method and fed for 30 days. On the 31st day of rearing, a challenge test was conducted by injecting *V. alginolyticus* intramuscularly at 10^6 CFU mL^{-1}. The results showed that *C. indicum* treatment for 30 days had a significantly different effect ($P<0.05$) on weight gain and average daily growth, while KVit and KAnt treatments were not different from the control. After the challenge test, all doses of *C. indicum* treatment, as well as KVit and KAnt treatments, showed survival significantly different ($P<0.05$) from that of the positive control, which ranged from 88.89% to 95.56% and was not significantly different ($P>0.05$) from the negative control. The survival rate condition is identical to the cumulative survival condition which shows the highest mortality found in the positive control. The administration of *C. indicum* at a dose of 5 g kg^{-1} to 20 g kg^{-1} improved the growth performance and prevented *V. alginolyticus* infection.

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1. Introduction

Asian seabass (*Lates calcarifer*, Bloch) is a commercially valuable aquaculture species in the Asia-Pacific region and Australia (Lim *et al*., 2021). However, diseases have become a major problem that cause significant economic loss, threatening the development of a sustainable Asian seabass culture (Mohamad *et al*., 2019a). Disease is one of the important problems in fish farming (Kusdarwati *et al*., 2016). One of the diseases affecting the fish is vibriosis caused by *Vibrio* groups, such as *Vibrio alginolyticus* (Sharma *et al*., 2012). Clinical signs of affected fish include weariness, anorexia, body discoloration, tail and fin rot, eye opacity, liquefaction of internal organs, hemorrhagic ulcers skin, and necrosis across vital organs (Ina-Salwany *et al*., 2019).

In general, antibiotics are used to prevent and control vibriosis in aquaculture (Abdel-Latif *et al*., 2022). However, the use of antibiotics may lead to an increased number of resistant bacteria and leave residues in the aquaculture organisms that could harm both humans and the aquatic environment (Liu *et al*., 2017; Lulijwa *et al*., 2020; Loo *et al*., 2020). The administration of medicinal plants in aquaculture for disease control is one of the promising alternative methods to the antibiotic's usage, towards an eco-friendly approach (Van Hai, 2015). This method offers several benefits. The materials are relatively affordable, it can be made by using simple methods, and are easily degradable, so they do not pollute the environment over a long term (Elumalai *et al*., 2020; Munaeni *et al*., 2020). In addition, medicinal plants can stimulate fish immunity and improve the growth performance of Asian seabass (Yang *et al*., 2020; Yu *et al*., 2022).

One of the medicinal plants known to have a good potency is *Canarium indicum* L. The plant is now commonly grown in Indonesia, Papua New Guinea, Solomon Islands, Vanuatu, Melanesia, Australia, Taiwan, Fiji, Hawaii, Honduras, and Trinidad (Lim, 2012). *C. indicum* has also been known as *C. amboinense* Hochreut., *C. commune* L., *C. grandistipulatum* Lauterb., *C. mehenbethene* Gaertn., *C. moluccanum* Blume, *C. nungi* Guill., *C. shortlandicum* Reching., *C. subtruncatum* Engl., and *C. zephyrinum* Rumphius (Lim, 2012).

This medicinal plant exhibits antibacterial (Indrianingsih *et al*., 2021) and anti-inflammatory properties (Leakey *et al*., 2008). It also has the potential to be a mercury antigenotoxic (Wibowo *et al*., 2019). Furthermore, *C. indicum* also contains vitamin E and vitamin C (Leakey *et al*., 2008; Lim, 2012), which functions as an antioxidant (Vinha *et al*., 2013; Carr and Maggini, 2017; Lee and Han, 2018). Previous studies have shown that vitamin E may improve the growth of Asian seabass (Jones and Carton, 2015) and resistance to *V. alginolyticus* in *Nibea albiflora* (Wang *et al*., 2019). Meanwhile, vitamin C supplementation in the *Rachycentron canadum* diet significantly affected growth, survival, and resistance to *V. harveyi* (Zhou *et al*., 2012).

However, no studies have been conducted on the use of *C. indicum* to improve Asian seabass growth performance, immunity, and resistance to diseases caused by pathogenic bacteria such as *V. alginolyticus*. This study aims to evaluate the administration of *C. indicum* as an immunostimulant through feed on the growth performance, immune responses, and resistance of Asian seabass, as an effort to prevent *V. alginolyticus* infection.

2. Materials and Methods

2.1 Experimental Design and Animals (Asian Seabass)

The containers used for rearing were 21 glass aquaria of size 60×30×30 cm^3. Each aquarium was equipped with a filter machine and an aerator. Water quality parameters such as temperature, salinity, pH, dissolved oxygen (DO), and total ammonia nitrogen (TAN) were measured at the beginning and end of the study from the experimental tanks. Water temperature, salinity, pH, DO, and TAN were checked using a digital thermometer, hand-held refractometer, portable pH meter, portable DO meter, and a spectrophotometer, respectively. The water quality parameters during the study were maintained at a temperature of 29.8–30.2°C, pH of 7.15–7.31, salinity of 15–20 g L^{-1}, dissolved oxygen (DO) of 5.8–6,5 mg L^{-1}, and total ammonia nitrogen (TAN) of < 0.66–0.98 mg L^{-1}. This water quality condition has met the requirements of the Indonesian National Standard (SNI 6145.4:2014).

Fingerlings of Asian seabass measuring 4.50±0.00 cm with average weight of 1.45±0.04 g was obtained from Aquaculture Production Business Service Center (BLUPPB Karawang) hatchery, Indonesia. The fingerlings used were free of vibriosis with Collagenase primers (Table 1) that have been certified by the Fish Health and Environmental Assessment Center (BPKIL Serang), Indonesia. The Asian seabass used for the treatments were stocked at a density of 15 fish per aquarium. Once a week, 50% of the water was replaced. During the experiment, total water volume in each tank was maintained uniformly, and aeration was provided

continuously. The present study fulfilled ethical requirements considering the welfare of the test animals and received ethical approval from the IPB University animal ethics commission (ethical approval number: 243–2022 IPB).

Table 1. Primer of the Collagenase genes used to identify *V. alginolyticus*

Gene	Primer		Amplicon (bp)	Ref.
Collagenase	VA-F	5'-CGAGTA-CAGTCACTT-GAAAGCC-3'	737	Di-Pinto *et al.* (2005)
	VA-R	5'-CACAA-CAGAACTCGC-GTTACC-3'		

2.2 Canarium indicum L. Collection

C. indicum fruits were collected from Makian Island, South Halmahera, Indonesia ($0^0$19'22,3" N and $127^0$24'2,43" E). The canarium nuts on the island have been described as *C. indicum* by Kamaluddin and Rasulu (2019). *C. indicum* fruits used were collected from trees that can grow at an altitude of 0-1000 meters above sea level with a loamy soil texture. The fruit part used for the study was the cotyledons, and the experimental feed was prepared at the Aquatic Organism Health Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Bogor, Indonesia.

2.3 Preparation of Treatment Diets

Treatment diets were prepared according to the methods previously described by Huang and Nitin (2019), with major modifications. The treatments consisted of negative control (K-), positive control (K+), 0.6 g kg^{-1} vitamin E administered (KVit), 2 g kg^{-1} chloramphenicol administered (KAnt), and three dosage levels of *C. indicum*; namely 5 g kg^{-1} (D05), 10 g kg^{-1} (D10), and 20 g kg^{-1} (D20). Vitamin E, chloramphenicol, and *C. indicum* cotyledons were first homogenized with water (30 mL kg^{-1}) and egg white (20 g kg^{-1}). The homogenized solution was then filtered and coated with commercial feed. The negative and positive control diets were also coated with water (30 mL kg^{-1}) and egg white (20 g kg^{-1}). The treatment diets were oven-dried for two hours and then stored at a room temperature in airtight jars labeled according to the treatment.

2.4 Vibrio alginolyticus Preparation

The *V. alginolyticus* used in this study was isolated from the culture collections BPKIL Serang. This isolation was made to increase its virulence according to the methods described by Ilmiah *et al.* (2012). The bacterium was cultured on thiosulfate citrate bile salt sucrose (TCBS) agar medium for 24 hours at 28°C and then transferred to 25 mL of complete seawater agar broth (SWC: 1 g of yeast extract, 3 mL of glycerol, 5 g of bactopeptone, 250 mL of distilled water, and 750 mL of seawater) for 18 hours at 29°C. The broth culture was centrifuged at 3500 rpm for five minutes. The supernatant was removed, the bacterial pellet was resuspended in phosphate-buffered saline (PBS) solution (8 g NaCl, 1.5 g Na_2HPO_4, 0.2 g KCl, 0.2 g KH_2PO_4, and 1000 mL distilled water), and then injected to Asian seabass at a dose of 100 µL $fish^{-1}$. The Asian seabass showing clinical signs of vibriosis were then isolated and identified with Collagenase primers (Table 1) at BPKIL Serang. The Asian seabass with clinical signs of vibriosis showed positive *V. alginolyticus* with PCR products measuring 737 bp (Figure 1).

2.5 Experimental Design and Observation Parameters

This study employed a completely randomized design with treatments K-, K+, KVit, KAnt, D05, D10, and D20, each consisting of three replicates. Before testing, 15 fish were taken from the stock to be used as the initial group on day 0 (without treatment). A feeding trial was done thrice a day (at 8 a.m., 12 p.m., and 5 p.m. Western Indonesia Time) for 30 days. *V. alginolyticus* challenge test was conducted on day 31 at a dose of 10^6 CFU mL^{-1} (the level found with an LD50 test) as 100 µL $fish^{-1}$, while KN fish were injected with PBS. After the challenge test, the test fish were reared for 14 days and fed commercial feed with 48% protein.

Different parameters of the experimental fish were estimated using standard procedures reported by earlier researchers. These include growth performance (Bunnoy *et al.*, 2022), health status, including total erythrocytes (Blaxhall and Daisley, 1973), total leukocytes (Blaxhall and Daisley, 1973), hematocrit level (Anderson and Siwicki, 1995), hemoglobin level (Wedemeyer and Yasutake, 1977), phagocytic activity (Anderson and Siwicki, 1995), respiratory burst activity (Anderson and Siwicki, 1995), lysozyme activity (Nayak *et al.*, 2023), clinical signs, histopathology liver (Izwar *et al.*, 2020), survival rate, and cumulative survival (Ghanei-Motlagh *et al.*, 2021). The growth performance was observed from day 0 to 30. The health status was observed on days 0 and 30 pre-challenge, days 1 post-

average final weight (Wt), weight gain (ΔW), and challenge (DPC), 3 DPC, 5 DPC, 7 DPC, and 14 DPC. Clinical signs, histopathology liver, survival rate, and cumulative survival were observed in the challenge test.

2.6 Analysis Data

The data were analyzed using Microsoft Excel 2010 and tested with analysis of variance (ANOVA). If there was a significant effect, Tukey's follow-up test was applied. Furthermore, clinical signs parameters were analyzed descriptively.

3. Results and Discussion

3.1 Results

3.1.1 Growth Performances

The growth performances at the end of the feeding trial showed significant differences in the average final weight (Wt), weight gain (ΔW), and average daily growth (ADG) ($P<0.05$) compared to those in the control (Table 2). The final weight, weight gain, and average daily growth in treatment D05, D10, and D20 were the highest compared to the treatments K, KVit, and KAnt. There were no significant differences ($P>0.05$) found in the survival rate (SR). At the same time, the average feed conversion ratio (FCR) was better in treatment K, which was significantly different from treatments KVit, KAnt, D05, D10, and D20 ($P<0.05$).

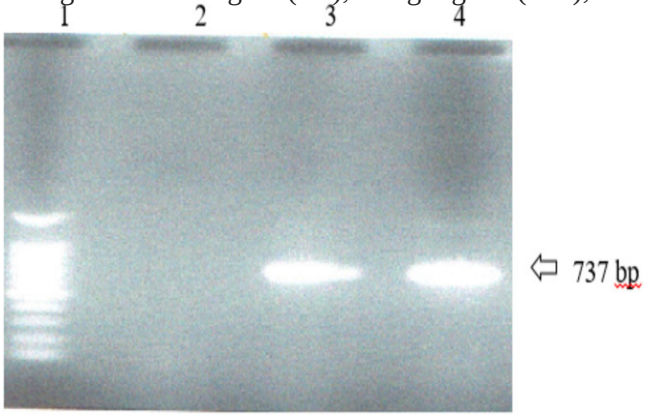

Figure 1. Agarose gel electrophoresis illustrates the duplex PCR results of the *V. alginolyticus* isolate. Lane 1 (marker), Lane 2 (negative control), Lane 3 (positive control), and Lane 4 (*V. alginolyticus* isolate).

3.1.2 Clinical Signs

Observation of the clinical signs revealed that the Asian seabass post-challenge *V. alginolyticus* has anorexia or decreased response to diet in treatments K+, KVit, KAnt, D05, D10, and D20 from the day 1 post-challenge, except in treatment K- (without *V. alginolyticus* injection). In addition, in the *V. alginolyticus* injected group, there were lesions in the operculum and abdominal dropsy since day 2 post-challenge (Figure 2).

3.1.3 Histopathologic Liver

Histopathology results for the liver from treatment K- showed hepatocytes cells in the normal liver. In addition, the infected liver tissues collected from treatments K+, KVit, KAnt, D05, D10, and D20 showed hemorrhage, vacuolization, and hepatocyte necrosis (Figure 3). The total histopathology score of the liver between treatments was significantly different ($P<0.05$) from the treatment K- (Table 3). After the challenge test, the treatment K- showed normal damage while the treatments K+, KVit, KAnt, D05, D10, and D20 showed mild damage.

Table 2. The initial weight (W0), final weight (Wt), weight gain (ΔW), average daily growth (ADG), survival rate (SR), and feed conversion ratio (FCR) (mean±SD) of Asian seabass fed treatment for 30 days

Parameter	Treatment					
	K	KVit	KAnt	D05	D10	D20
W0 (g)	1.41±0.04^{a}	1.40±0.04^{a}	1.40±0.04^{a}	1.40±0.04^{a}	1.41±0.03^{a}	1.40±0.04^{a}
Wt (g)	12.36±0.04^{a}	12.41±0.07^{ab}	12.30±0.06^{a}	12.62±0.09^{b}	12.64±0.07^{b}	12.73±0.10^{b}
ΔW (g)	10.95±0.06^{a}	11.01±0.04^{ab}	10.89±0.08^{a}	11.21±0.05^{b}	11.23±0.09^{b}	11.33±0.13^{b}
ADG (g day^{-1})	0.37±0.00^{a}	0.37±0.00^{ab}	0.36±0.00^{a}	0.37±0.00^{b}	0.37±0.00^{b}	0.38±0.00^{b}
SR (%)	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}
FCR	1.74±0.01^{a}	1.78±0.00^{b}	1.93±0.01^{c}	1.78±0.01^{b}	1.79±0.01^{b}	1.78±0.02^{b}

Description: K (control), KVit (vitamin E control), KAnt (antibiotics control), D50 (5 g kg^{-1} *C. indicum*), D10 (10 g kg^{-1} *C. indicum*), and D20 (15 g kg^{-1} *C. indicum*). Different superscripts in the same line with different letters indicate significantly different ($P<0.05$).

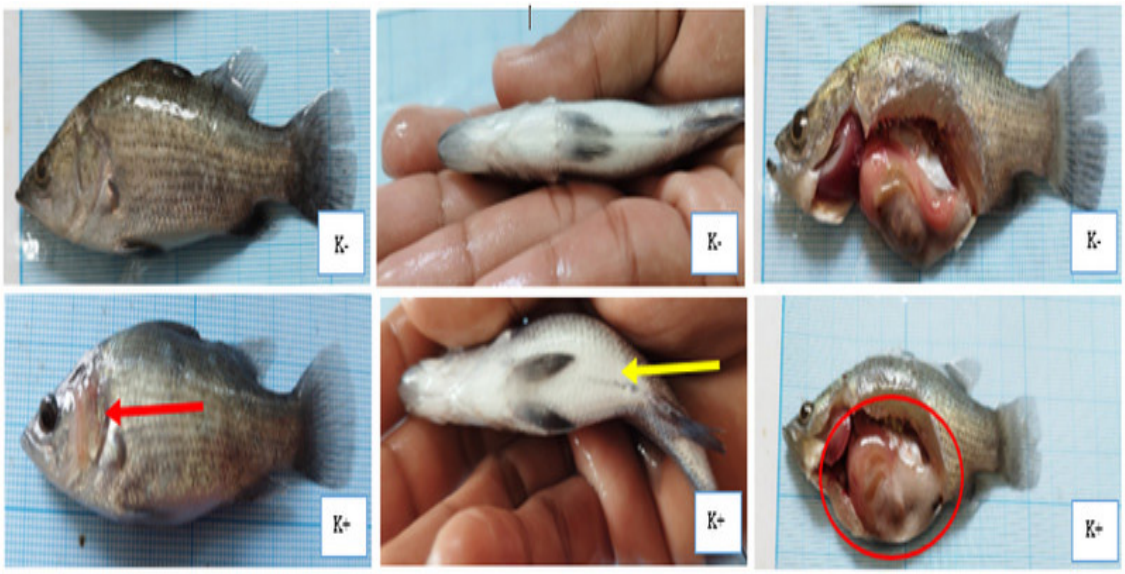

Figure 2. The clinical signs after the challenge tests. red arrow (lesions in the operculum), yellow arrow (abdominal dropsy), red circle (abdominal dropsy).

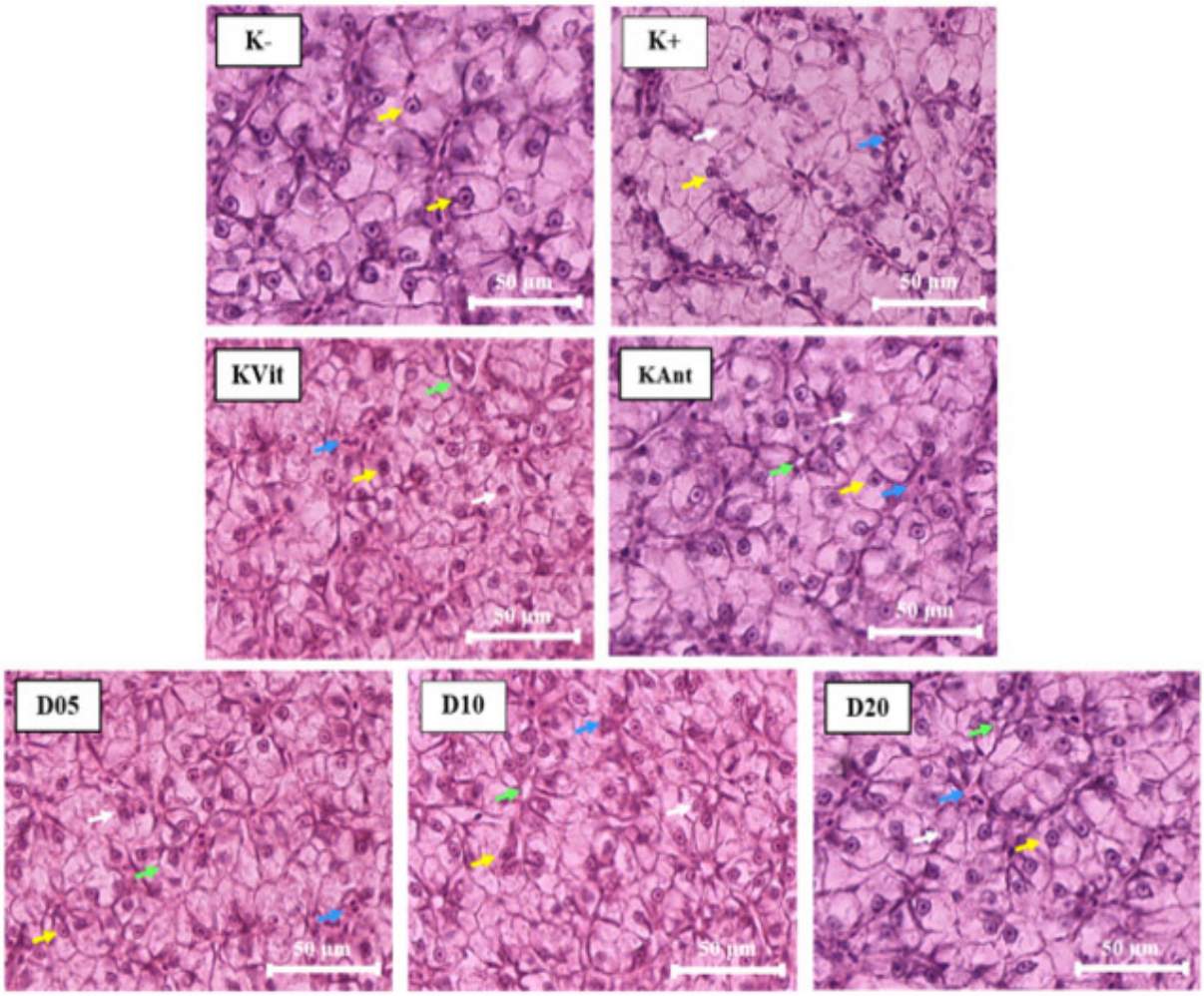

Figure 3. Liver tissue condition of the Asian seabass after the challenge test. K- (negative control), K+ (positive control), KVit (vitamin E control), KAnt (antibiotic control), D05 (5 g kg^{-1} *C. indicum*), D10 (10 g kg^{-1} *C. indicum*), D20 (20 g kg^{-1} *C. indicum*), yellow arrows (normal hepatocyte cells), blue arrows (hemorrhage), green arrows (vacuolization), white arrows (hepatocyte necrosis).

Table 3. Total histopathology score (mean±SD) of Asian seabass liver after challenge test

Treatment	Histopathologic score	Degrees of damage
K-	1.00±0.00[a]	Normal
K+	2.00±0.00[b]	Mild damage
KVit	1.67±0.58[b]	Mild damage
KAnt	2.00±0.00[b]	Mild damage
D05	2.00±0.00[b]	Mild damage
D10	2.00±0.00[b]	Mild damage
D20	2.00±0.00[b]	Mild damage

Description: K- (negative control), K+ (positive control), KVit (vitamin E control), KAnt (antibiotics control), D50 (5 g kg^{-1} *C. indicum*), D10 (10 g kg^{-1} *C. indicum*), and D20 (15 g kg^{-1} *C. indicum*). Different superscripts in the same line with different letters indicate significantly different (P<0.05).

3.1.4 Hematological

Total erythrocytes on day 0 and day 30 were not significantly different (P>0.05) between treatments (Table 4). Total erythrocytes on day 1 and day 5 after the challenge test decreased in all *V. alginolyticus* injection treatments (K+, KVit, KAnt, D05, D10, and D20) which were significantly different (P<0.05) from the negative control (K-). Meanwhile. on day 14 post-challenge test, total erythrocytes increased in all *V. alginolyticus* injection treatments and were not significantly different (P>0.05) from the negative control treatment.

Total leukocytes on days 0 and 30 were not significantly different (P>0.05) between treatments (Table 4). Total leukocytes on day 1 and day 5 after the challenge test increased in all *V. alginolyticus* injection treatments, showing a significant difference (P<0.05) compared to the negative control treatment. Meanwhile, on day 14 after the challenge test, total leukocytes decreased in all *V. alginolyticus* injection treatments and were not significantly different (P>0.05) from the negative control.

Hematocrit levels on days 0 and 30 were not significantly different (P>0.05) between treatments (Table 4). On day 1 after the challenge test, there was a decrease in hematocrit levels in the *V. alginolyticus* injection treatment but not significantly different (P>0.05) from the negative control treatment. In contrast, it showed a significant difference on day 5 after the challenge test (P<0.05). Hematocrit levels increased on day 14 after the challenge test in all *V. alginolyticus* injection treatments and were not significantly different (P>0.05) from the negative control.

Hemoglobin conditions on days 0 and 30 were not significantly different (P>0.05) between treatments (Table 4). Hemoglobin on day 1 and day 5 after the challenge test decreased in all *V. alginolyticus* injection treatments and showed a significant difference (P<0.05) from the negative control treatment. Meanwhile, on day 14 after the challenge test, hemoglobin increased in all *V. alginolyticus* injection treatments and was not significantly different (P>0.05) from the negative control.

3.1.5 Immune response

Phagocytic activity on day 0 was not significantly different (P>0.05) between treatments (Figure 4), while it was significantly different on day 30 (P<0.05). Phagocytic activity on day 1 and day 5 after the challenge test increased in all *V. alginolyticus* injection treatments showed a significant difference (P<0.05) compared to the negative control treatment. Meanwhile, on day 14 after the challenge test, phagocytic activity decreased again in all *V. alginolyticus* injection treatments and was not significantly different (P>0.05) from the negative control.

Respiratory burst activity on day 0 was not significantly different (P>0.05) between treatments (Figure 4), while it was significantly different on day 30 (P<0.05). Respiratory burst activity on day 1 and day 5 after the challenge test increased in all *V. alginolyticus* injection treatments and was significantly different (P < 0.05) from the negative control treatment. Meanwhile, on day 14 after the challenge test, respiratory burst activity decreased again in all *V. alginolyticus* injection treatments and was not significantly different (P>0.05) from the negative control.

Lysozyme activity on days 0 and 30 was not significantly different (P>0.05) between treatments (Figure 4). Lysozyme activity on day 1 and day 5 after the challenge test increased in all *V. alginolyticus* injection treatments and was significantly different (P<0.05) from the negative control treatment. Meanwhile, on day 14 after the challenge test, lysozyme activity decreased again in all *V. alginolyticus* injection treatments and was not significantly different (P>0.05) from the negative control treatment. Lysozyme activity on days 0 and 30 were not significantly different (P>0.05) between treatments.

3.1.6 Survival rate and cumulative survival post-challenge test

The survival value shows that treatment

Table 4. The hematology (mean±SD) of Asian seabass pre-challenge (days 0 and 30 pre-challenge) and post-challenge (1 DPC, 3 DPC, 5 DPC, 7 DPC, and 14 DPC)

Parameter	Treatment	Periods				
		D0	D30	D+1	D+5	D+14
Erythrocyte	K-	3.12±0.02^{a}	3.26±0.02^{a}	3.27±0.02^{b}	3.26±0.01^{c}	3.25±0.01^{a}
(10^{6} cell mm^{-3})	K+	3.12±0.02^{a}	3.26±0.02^{a}	3.19±0.01^{a}	3.04±0.02^{a}	3.24±0.01^{a}
	KVit	3.12±0.02^{a}	3.25±0.02^{a}	3.20±0.02^{a}	3.07±0.02^{ab}	3.24±0.02^{a}
	KAnt	3.12±0.02^{a}	3.26±0.02^{a}	3.21±0.02^{a}	3.09±0.01^{b}	3.24±0.01^{a}
	D05	3.12±0.02^{a}	3.25±0.01^{a}	3.21±0.02^{a}	3.07±0.02^{ab}	3.25±0.02^{a}
	D10	3.12±0.02^{a}	3.26±0.01^{a}	3.21±0.02^{a}	3.08±0.01^{b}	3.25±0.01^{a}
	D20	3.12±0.02^{a}	3.26±0.01^{a}	3.22±0.02^{a}	3.08±0.02^{b}	3.24±0.02^{a}
Leucocytes (10^{5} cell mm^{-3})	K-	8.20±0.17^{a}	9.47±0.06^{a}	8.33±0.15^{a}	8.50±0.17^{a}	8.60±0.17^{a}
	K+	8.20±0.17^{a}	9.30±0.10^{a}	9.00±0.1^{b}	9.80±0.17^{b}	8.50±0.17^{a}
	KVit	8.20±0.17^{a}	9.17±0.15^{a}	9.10±0.17^{b}	10.23±0.12^{bc}	8.57±0.15^{a}
	KAnt	8.20±0.17^{a}	9.30±0.10^{a}	9.17±0.06^{b}	10.40±0.17^{c}	8.47±0.15^{a}
	D05	8.20±0.17^{a}	9.33±0.06^{a}	9.17±0.15^{b}	10.03±0.12^{bc}	8.50±0.10^{a}
	D10	8.20±0.17^{a}	9.40±0.10^{a}	9.17±0.15^{b}	10.10±0.17^{bc}	8.57±0.12^{a}
	D20	8.20±0.17^{a}	9.37±0.21^{a}	9.70±0.17^{c}	10.30±0.17^{c}	8.43±0.15^{a}
Hematocrit (%)	K-	25.03±0.45^{a}	25.15±1.13^{a}	25.04±1.02^{a}	24.98±1.51^{b}	24.50±0.44^{a}
	K+	25.03±0.45^{a}	25.51±0.50^{a}	23.25±1.36^{a}	20.85±1.34^{a}	25.00±0.45^{a}
	KVit	25.03±0.45^{a}	25.07±0.81^{a}	24.03±1.03^{a}	21.14±0.83^{a}	24.78±0.45^{a}
	KAnt	25.03±0.45^{a}	25.02±0.32^{a}	23.97±0.94^{a}	21.43±0.27^{a}	24.78±0.45^{a}
	D05	25.03±0.45^{a}	25.54±0.72^{a}	24.20±1.12^{a}	20.88±1.13^{a}	24.75±0.45^{a}
	D10	25.03±0.45^{a}	24.93±0.38^{a}	24.27±1.46^{a}	21.04±1.52^{a}	24.75±0.45^{a}
	D20	25.03±0.45^{a}	25.21±0.57^{a}	24.20±1.08^{a}	21.02±1.14^{a}	24.75±0.45^{a}
Haemoglobin (g dL^{-1})	K-	8.20±0.35^{a}	8.00±0.20^{a}	8.40±0.20^{b}	8.27±0.31^{b}	8.13±0.42^{a}
	K+	8.20±0.35^{a}	8.27±0.31^{a}	6.40±0.60^{a}	3.87±1.01^{a}	8.33±0.42^{a}
	KVit	8.20±0.35^{a}	8.27±0.46^{a}	7.00±0.20^{a}	4.67±1.10^{a}	7.80±0.72^{a}
	KAnt	8.20±0.35^{a}	8.33±0.12^{a}	7.40±0.20^{a}	4.47±1.14^{a}	7.93±0.12^{a}
	D05	8.20±0.35^{a}	8.33±0.31^{a}	7.20±1.06^{a}	5.07±0.61^{a}	8.13±0.31^{a}
	D10	8.20±0.35^{a}	8.27±0.31^{a}	7.33±0.76^{a}	6.07±1.21^{a}	8.20±0.53^{a}
	D20	8.20±0.35^{a}	8.33±0.31^{a}	7.40±1.25^{a}	5.40±01.40^{a}	7.67±0.42^{a}

Description: K- (negative control), K+ (positive control), KVit (vitamin E control), KAnt (antibiotics control), D50 (5 g kg^{-1} *C. indicum*), D10 (10 g kg^{-1} *C. indicum*), and D20 (15 g kg^{-1} *C. indicum*). Different superscripts in the same line with different letters indicate significantly different (P<0.05).

D05, D10, and D20 in diet has higher results and is significantly different (P<0.05) from the K+ treatment (Figure 5). On the other hand, the treatment of *C. indicum* was not significantly different (P>0.05) from the K-, KVit, and KAnt. The survival rate condition is identical to the cumulative survival condition which shows the highest mortality found in the K+ treatment (Figure 6).

3.2 Discussion

The sustainability of Asian seabass aquaculture production is affected by various constraints. The attack of Vibriosis disease is one of the main obstacles that can harm aquaculture businesses (Mohamad *et al*., 2019a). *V. alginolyticus* is one species that causes vibriosis in Asian seabass (Sharma *et al*., 2012). Vibriosis control can be done by administering antibiotics. However, this method can harm the aquatic environment and consumer health and cause antibiotic resistance as the causative agent of vibriosis (Liu *et al*., 2017; Lulijwa *et al*., 2020; Loo *et al*., 2020). Applying herbal ingredients is an alternative to antibiotic substitution in aquaculture activities (Van Hai, 2015).

Applying herbal ingredients in fish farming activities can significantly improve growth performance (Huang *et al*., 2022). This study showed that *C. indicum* significantly increased the growth of Asian seabass (Table 2). *C. indicum* has a 17-34 mg g^{-1} vitamin E and 8 mg g^{-1} vitamin C (Leakey *et al*., 2008; Lim, 2012). These components are expressed as cofactors of digestive enzymes, such as succinic acid dehydrogenase, acid phosphatase, glucose 6 phosphatase, and adenosine triphosphate (Islam *et al*., 2021). Increased digestive enzymes in fish fed with vitamin E and C diet will have implications for increased fish growth (Liu *et al*., 2019).

In addition, canarium also contains polyphenolic compounds such as tannins and flavonoids that can improve gut microbiota and act as prebiotics for *Lactobacillus bulgaricus* and *S. thermophilus* (Wen *et al*., 2013; Zhang *et al*., 2018). The presence of these probiotics in the digestive tract can increase enzyme activity, further enhancing fish growth (Mohammadian *et al*., 2022; Keereelang *et al*., 2022). Therefore, *C. indicum* can significantly increase the growth of Asian seabass. This aligns with previous studies which revealed that canarium could increase growth in catfish *C. gariepinus*, Broiler, and rat *Rattus novergicus* (Ananias *et al*., 2014; Sokoudjou *et al*., 2019; Mailoa *et al*., 2019).

Applying herbal ingredients in fish farming also significantly increases the resistance to vibriosis disease attacks (Yu *et al*., 2022). The *C. indicum* treatment had a higher and cumulative survival (Figure 5 and Figure 6) values than the positive control treatment during the *V. alginolyticus* challenge test. The treatments challenged with *V. alginolyticus* showed clinical signs such as anorexia, lesions operculum, dropsy abdomen, dropsy internal organ (Figure 2), and death. These clinical signs revealed that vibriosis in fish is characterized by behavioral changes such as anorexia (Ransangan and Mustafa, 2009), lesions operculum (Turgay and Karataş, 2016), dropsy abdomen (Canak and Akayli, 2018), and dropsy internal organ (Silvaraj *et al*., 2020).

Histopathologically (Figure 3), *V. alginolyticus* infection also resulted in the changes of Asian seabass tissue condition. Histological features of Asian seabass' liver challenged with *V. alginolyticus* in this study showed a hemorrhage, vacuolization, and hepatocyte necrosis. This condition is identical to that found by Sumithra *et al*. (2022), that pathogen infection in Asian seabass causes changes in the structure of the liver tissue showing hemorrhage, vacuolization, and hepatocyte necrosis. Further observation using the scoring method (Table 3) revealed that after the challenge test, the treatment injected with *V. alginolyticus* showed a mild damage to the liver, while the negative control treatment showed a normal damage. The mild damage found in this study is the same as Mohamad *et al*. (2019b) reported on grouper hybrid *Epinephelus polyphekadion* x *E. Fuscoguttatus* injected with *V. alginolyticus*.

V. alginolyticus infection can cause the death of Asian seabass, with mortality rates ranging from 40% to 100% (Ransangan *et al*., 2012). *V. alginolyticus* can produce several toxins and virulence factors that cause death in the host (Liu *et al*., 2020). Hemolysin is one of the virulence factors produced by *V. alginolyticus* and can result in hemolysis, which is the destruction of red blood cell walls (Wang *et al*., 2007). In line with this, the condition of total erythrocytes after the challenge test decreased the number of cells in the *V. alginolyticus* injection treatment, which was significantly different from the negative control treatment (Table 4). The value of total erythrocytes in the negative control was at the standard concentration for Asian seabass which was 10^6 cells mm^{-3} (Ali *et al*., 2017; Pattah *et al*., 2021).

This condition of the total erythrocyte parameter also affects hematocrit and hemoglobin, as these parameters have a strong correlation. The decrease in erythrocytes also caused a decrease in hematocrit and hemoglobin of Asian seabass after the challenge test. In addition, the decrease in hemoglobin levels is also indicated by the influence of the virulence factor *V. alginolyticus* siderophore which can bind iron

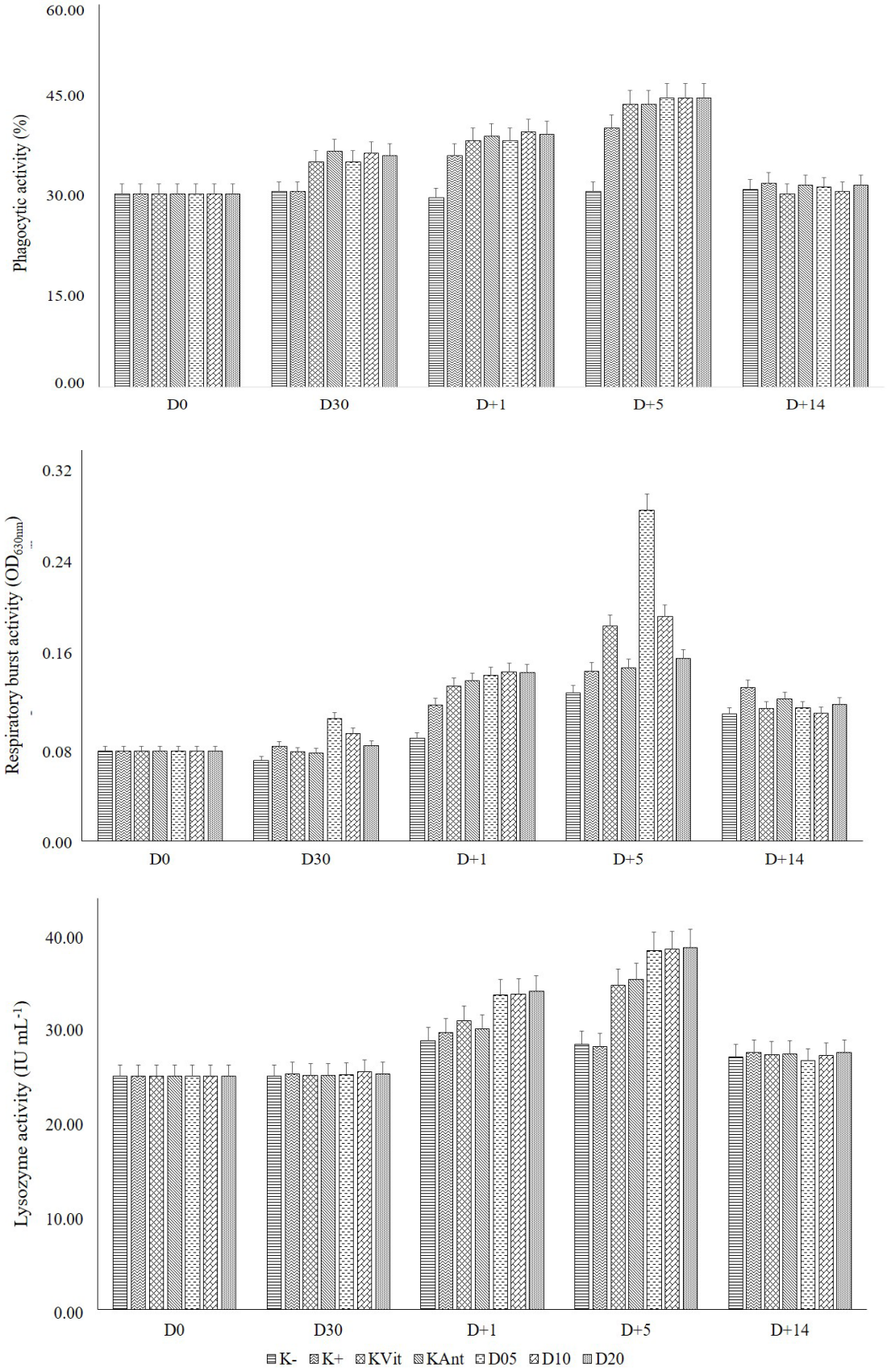

Figure 4. Immune response parameters of Asian seabass pre-challenge (days 0 and 30 pre-challenge) and post-challenge (1 DPC, 3 DPC, 5 DPC, 7 DPC, and 14 DPC). K- (negative control), K+ (positive control), KVit (vitamin E control), KAnt (antibiotic control), D05 (5 g kg^{-1} *C. indicum*), D10 (10 g kg^{-1} *C. indicum*), D20 (20 g kg^{-1} *C. indicum*).

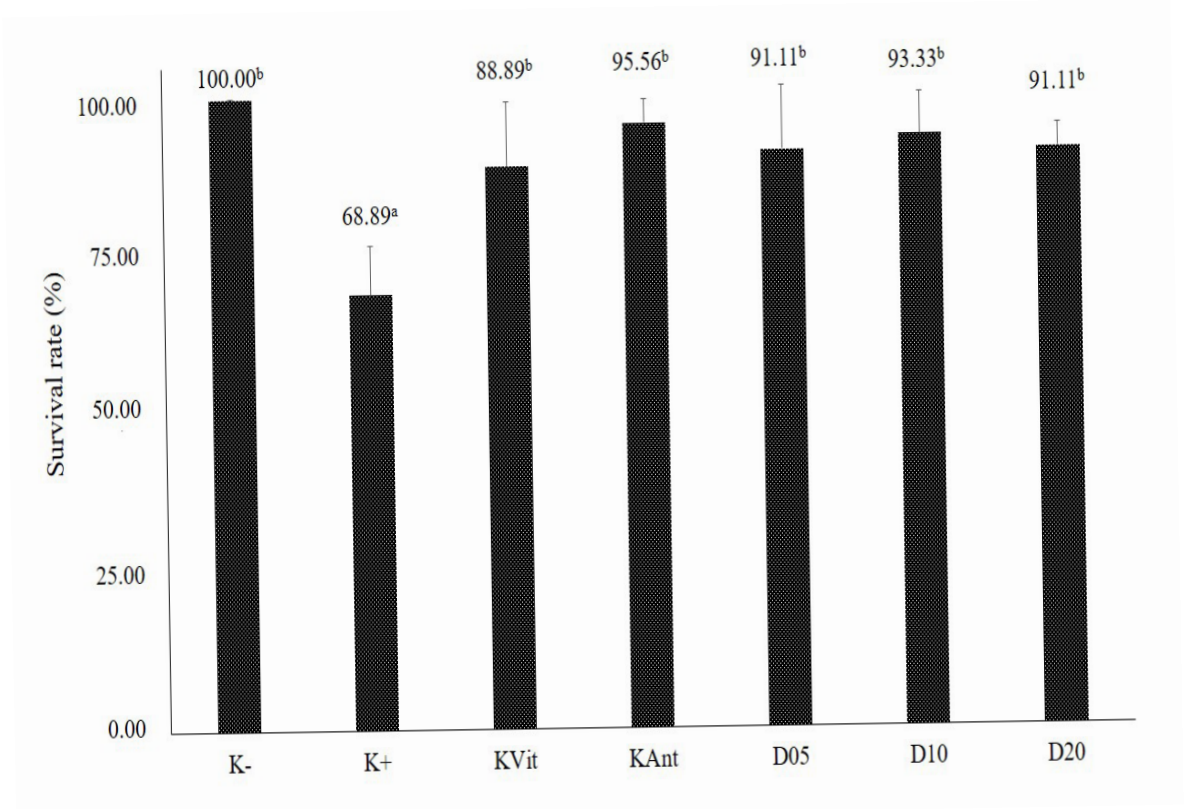

Figure 5. Survival rate on the Asian seabass post-challenge test. Data are mean ± SD. K- (negative control), K+ (positive control), KVit (vitamin E control), KAnt (antibiotic control), D05 (5 g kg^{-1} *C. indicum*), D10 (10 g kg^{-1} *C. indicum*), D20 (20 g kg^{-1} *C. indicum*).

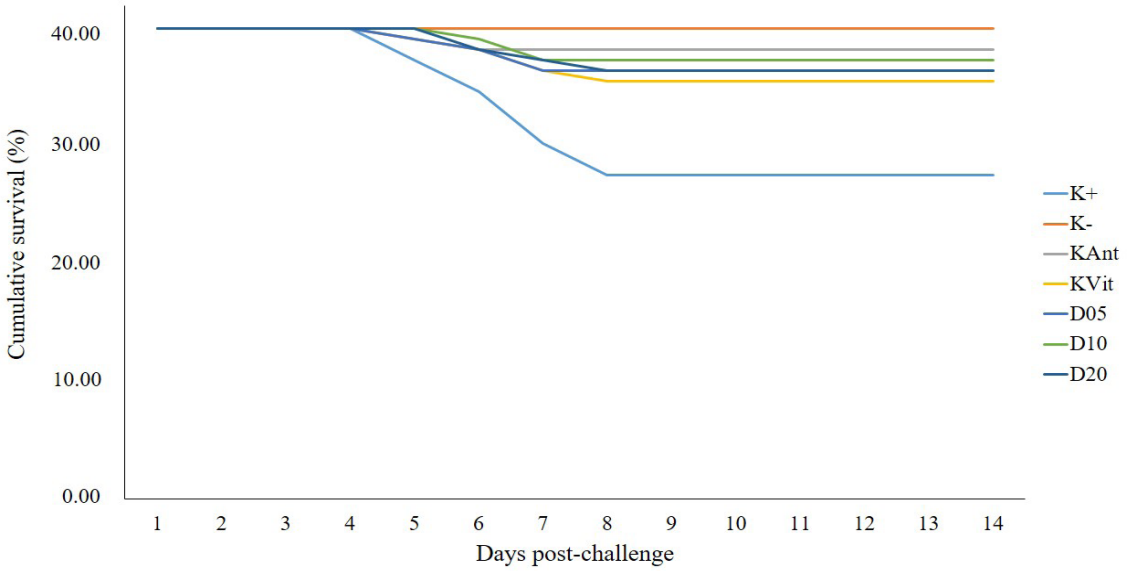

Figure 6. Cumulative survival on the Asian seabass post-challenge test. K- (negative control), K+ (positive control), KVit (vitamin E control), KAnt (antibiotic control), D05 (5 g kg^{-1} *C. indicum*), D10 (10 g kg^{-1} *C. indicum*), D20 (20 g kg^{-1} *C. indicum*).

to hemoglobin (Wang *et al*., 2007). In line with this, the condition of hematocrit and hemoglobin after the challenge test decreased the number of cells in the *V. alginolyticus* injection treatment, showing a significant difference from the negative control treatment (Table 4). The hematocrit and hemoglobin values in the negative control were at the normal concentrations for Asian seabass which were 18% to 25% (Talpur and Ikhwanuddin, 2012; Talpur *et al*., 2013) and 6 to 8 g dL^{-1} (Ali *et al*., 2017; Pattah *et al*., 2021), respectively. The results of total erythrocytes, hematocrit, and hemoglobin levels in this study are identical to those found in silver sea bream when injected with *V. alginolyticus* (Li *et al*., 2003).

V. alginolyticus infection also affects the immune system in fish (Lim *et al*., 2021). Leukocytes are one of the body's responses that will increase due to foreign body intervention, such as bacteria (Tadese *et al*., 2022). In connection with this, the total leukocytes experienced an increase in the number of cells after the challenge test showed a significant difference compared to the negative control treatment (Table 4). The value of total leukocytes in the negative control is at the normal concentration for Asian seabass which is 10^5 cells mm^{-3} (Pattah *et al*., 2021). One of the mechanisms of leukocyte resistance to bacterial infection is phagocytosis through phagocytic cells such as neutrophils, monocytes, and macrophages so that there will be an increase in phagocytic activity (Abarike *et al*., 2019). Therefore, in this study, there was also an increase in phagocytic activity after the challenge test, which was significantly different from the negative control treatment (Figure 4). The phagocytic activity value in the negative control is at a normal concentration for Asian seabass which is 25% to 82% (Talpur *et al*., 2013; Lim *et al*., 2021).

Indeed, when phagocytosis occurs, phagocytic cells produce reactive oxygen species (ROS). Producing ROS is referred to as respiratory burst activity (Bandeira Junior and Baldisserotto, 2021). Reactive Oxygen Species is one of the lethal chemicals that can eliminate bacteria. The state of increased phagocytic activity will be directly proportional to respiratory burst activity (Rawling *et al*., 2012). The respiratory burst activity of Asian seabass increased after the challenge test, which was significantly different from the negative control treatment (Figure 4). The phagocytic activity value in the negative control was at the normal concentration for Asian seabass, which was 0.2 to 0.6 OD_{630nm} (Lim *et al*., 2021; Pattah *et al*., 2021).

In addition to producing ROS, phagocytic cells, especially neutrophils and macrophages, produce lysozyme (Uribe-Querol and Rosales, 2020). Lysozyme is a mucolytic enzyme that has a dual role in damaging bacterial cells, lysing the peptidoglycan of the bacterial cell wall, and activating autolytic enzymes in bacterial cells (Ganz, 2006). Therefore, the increased phagocytic activity will also impact the lysozyme activity (Dotta *et al*., 2014). The lysozyme activity of Asian seabass increased after the challenge test, showing a significant difference from the negative control treatment (Figure 4). The value of lysozyme activity in the negative control is normal for Asian seabass, which is 23 to 209 IU mL^{-1} (Lim *et al*., 2021; Pattah *et al*., 2021). The results of total leukocytes, phagocytic activity, respiratory burst, and lysozyme in this study are identical to those found by Lim *et al*. (2021) and Pattah *et al*. (2021) in Asian seabass injected with *V. alginolyticus*.

Observational data on total erythrocytes and leukocytes, hematocrit levels, hemoglobin, phagocytic activity, respiratory burst activity, and lysozyme activity revealed that *C. indicum* in feed could prevent *V. alginolyticus* infection. After the challenge test, the *C. indicum* treatment showed that the concentrations of total erythrocytes and leukocytes, hematocrit levels, hemoglobin, phagocytic activity, respiratory burst activity, and lysozyme activity were better than those in the positive control treatment. *C. indicum* can prevent *V. alginolyticus* infection because it helps the immune system function as an exogenous antioxidant (Apsari *et al*., 2023). In addition, vitamin E, vitamin C, and polyphenolic compounds also act as antioxidants (Carr and Maggini, 2017; Lee and Han, 2018; Rudrapal *et al*., 2022).

V. alginolyticus infection is well known to induce the body in order to produce an excessive amount of ROS such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) in the host (Mao *et al*., 2021). Excessive amounts of ROS can damage important biological molecules such as nucleic acids, proteins, and lipids in fish (Biller and Takahashi, 2018). Naturally, the fish body has defense mechanisms against ROS, namely endogenous antioxidants in the form of enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione-s-transferase (GST), and glutathione peroxidase (GPx) (Garai *et al*., 2021). However, when bacterial infection and oxidative stress occur simultaneously, endogenous antioxidants' performance will decrease, as Adeyemi (2014) reported in catfish *C. gariepinus* when challenged with *E. coli* and *V. fischeri* under oxidative stress conditions.

Therefore, the *C. indicum* treatment had a higher and cumulative survival (Figure 5 and Figure 6) values than the positive control treatment during the *V. alginolyticus* challenge test. This shows that *C. indicum*

can protect Asian seabass from disease attacks, which is in line with the research of Sokoudjou *et al.* (2019) who revealed that Canarium could control salmonellosis disease due to *S. enterica* infection in Broiler.

4. Conclusion

The administration of *C. indicum* at doses of 5 g kg^{-1} to 20 g kg^{-1} improves the growth performance and prevents *V. alginolyticus* infection because it can produce better survival and cumulative survival than the positive control treatment. Therefore, it can be used as an immunostimulant in Asian seabass.

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Authors' Contributions

The contribution of each author is as follows, TFA; collected the data, drafted the manuscript, and designed the table as well as the graph. DMW and W; devised the main conceptual ideas and conducted a critical revision of the article. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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References

Abarike, E. D., Kuebutornye, F. K. A., Jian, J., Tang, J., Lu, Y., & Cai, J. (2019). Influences of immunostimulants on phagocytes in cultured fish: A mini review. *Reviews in Aquaculture*, 11(4):1219–1227.

Abdel-Latif, H. M. R., Yilmaz, E., Dawood, M. A. O., Ringø, E., Ahmadifar, E., & Yilmaz, S. (2022). Shrimp vibriosis and possible control measures using probiotics, postbiotics, prebiotics, and synbiotics: A review. *Aquaculture*, 551:737951.

Adeyemi, J. A. (2014). Oxidative stress and antioxidant enzymes activities in the African catfish, *Clarias gariepinus*, experimentally challenged with *Escherichia coli* and *Vibrio fischeri*. *Fish Physiology and Biochemistry*, 40:347–354.

Ali, S. S. R., Ambasankar, K., Musthafa, M. S., & Harikrishnan, R. (2017). *Jerusalem artichoke* enriched diet on growth performance, immuno-hematological changes and disease resistance against *Aeromonas hydrophila* in Asian seabass (*Lates calcarifer*). *Fish & Shellfish Immunology*, 70:335–342.

Ananias, T. Y., Abdullahi, O. M., & Yusuf, G. A. (2014). Growth performance and body composition of *Clarias gariepinus* fingerlings fed varying inclusion level of *Canarium schweinfurtii* (African Elemi) oil. Paper presented at the Conference: International Institute of Chemical , Biological, and Environmental Engineering, Dubai, UAE.

Anderson, D. P., & Siwicki, A. (1995). Basic hematolog and serology for fish health. Manila: Fish Health Section, Asian Fisheries Society.

Apsari, C. N., Nugrahani, I., Sukrasno, & Gusdinar, T. (2023). Optimization of active antioxidative defatted *Canarium indicum* L. (Canary) protein hydrolysate production. *Journal of Applied Biology and Biotechnology*, 11(2):187–197.

Bandeira Junior, G., & Baldisserotto, B. (2021). Fish infections associated with the genus *Aeromonas*: a review of the effects on oxidative status. *Journal of Applied Microbiology*, 131(3):1083–1101.

Biller, J. D., & Takahashi, L. S. (2018). Oxidative stress and fish immune system: phagocytosis and leukocyte respiratory burst activity. *Anais da Academia Brasileira de Ciências*, 90:3403–3414.

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

Bunnoy, A., Thangsunan, P., Chokmangmeepisarn, P., Yata, T., Klongklaew, N., Pirarat, N., Kitiyodom, S., Srisapoome, P., & Rodkhum, C. (2022). Mucoadhesive cationic lipid-based *Flavobacterium oreochromis*

nanoencapsulation enhanced the efficacy of mucoadhesive immersion vaccination against *columnaris* disease and strengthened immunity in Asian sea bass (*Lates calcarifer*). *Fish & Shellfish Immunology*, 127:633–646.

Canak, O., & Akayli, T. (2018). Bacteria recovered from cultured gilt-head seabream (*Sparus aurata*) and their antimicrobial susceptibilities. *European Journal of Biology*, 77(1):11–17.

Carr, A. C., & Maggini, S. (2017). Vitamin C and immune function. *Nutrients*, 9(11):1211.

Di Pinto, A., Ciccarese, G., Tantillo, G., Catalano, D., & Forte, V. T. (2005). A collagenase-targeted multiplex PCR assay for identification of *Vibrio alginolyticus*, *Vibrio cholerae*, and *Vibrio parahaemolyticus*. *Journal of Food Protection*, 68(1):150–153.

Dotta, G., de Andrade, J. I. A., Gonçalves, E. L. T., Brum, A., Mattos, J. J., Maraschin, M., & Martins, M. L. (2014). Leukocyte phagocytosis and lysozyme activity in Nile tilapia fed supplemented diet with natural extracts of propolis and *Aloe barbadensis*. *Fish & Shellfish Immunology*, 39(2):280–284.

Elumalai, P., Kurian, A., Lakshmi, S., Faggio, C., Esteban, M. A., & Ringø, E. (2020). Herbal immunomodulators in aquaculture. *Reviews in Fisheries Science & Aquaculture*, 29(1):33–57.

Ganz, T. (2006). Lysozyme. *Encyclopedia of Respiratory Medicine*, 649–653.

Garai, P., Banerjee, P., Mondal, P., & Saha, N. C. (2021). Effect of heavy metals on fishes: Toxicity and bioaccumulation. *Journal of Clinical Toxicology*, 11(S18):1–10.

Ghanei-Motlagh, R., Gharibi, D., Mohammadian, T., Khosravi, M., Mahmoudi, E., Zarea, M., Menanteau-Ledouble, S., & El-Matbouli, M. (2021). Feed supplementation with quorum quenching probiotics with anti-virulence potential improved innate immune responses, antioxidant capacity and disease resistance in Asian seabass (*Lates calcarifer*). *Aquaculture*, 535:736345.

Huang, H. T., Lee, P. T., Liao, Z. H., Huang, C. T., Chen, H. Y., Huang, Z. G., Nan, F. H., & Wu, Y. S. (2022). Raw garlic (*Allium sativum*) improves nonspecific immune responses and resistance against *Vibrio alginolyticus* infection in grouper (*Epinephelus coioides*). *Journal of Marine Science and Technology*, 29(6):757–766.

Huang, K., & Nitin, N. (2019). Edible bacteriophage based antimicrobial coating on fish feed for enhanced treatment of bacterial infections in aquaculture industry. *Aquaculture*, 502:18–25.

Ilmiah, Sukenda, Widanarni, & Harris, E. (2012). Isolation and characterization of pathogenic Vibrio on tiger grouper *Epinephelus fuscoguttatus*. *Jurnal Akuakultur Indonesia*, 11(1):28–37.

Ina-Salwany, M. Y., Al-saari, N., Mohamad, A., Mursidi, F. A., Mohd-Aris, A., Amal, M. N. A., Kasai, H., Mino, S., Sawabe, T., & Zamri-Saad, M. (2019). Vibriosis in fish: a review on disease development and prevention. *Journal of Aquatic Animal Health*, 31(1):3–22.

Indrianingsih, A. W., Rosyida, V. T., Apriyana, W., Darsih, C., Angwar, M., Windarsih, A., & Batrisya. (2021). The antioxidant and antibacterial study of *Canarium indicum* L. latex extract. IOP Conf Series: *Materials Science and Engineering,* 1011:012051.

Islam, M. J., Kunzmann, A., Henjes, J., & Slater, M. J. (2021). Can dietary manipulation mitigate extreme warm stress in fish? The case of European seabass, *Dicentrarchus labrax*. *Aquaculture*, 545:737153.

Izwar, A., Nuryati, S., Rahman, & Purnomowati, R. (2020). Isolation, identification, and pathogenicity tests of pathogenic bacterial associated with black body syndrome in white barramundi Lates calcarifer B. *Jurnal Akuakultur Indonesia*, 19(1):39–49.

Jones, B. C., & Carton, A. G. (2015). Effects of dietary enrichment with alpha-tocopherol acetate and post-harvest filleting on lipid oxidation and flesh quality of tropical farmed barramundi (*Lates calcarifer*). *Aquaculture,* 448:280–287.

Kamaluddin, A. K., & Rasulu, H. (2019). Identification

of non timber forest product use of canarium nut (*Canarium* Sp) in Makian Island. IOP Conference Series: *Earth and Environmental Science,* 347(1):012033.

Keereelang, J., Mangumphan, K., Chitmanat, C., Tongsiri, S., Linh, N. V., & Van Doan, H. (2022). Dietary effect of *Lactobacillus plantarum* (TISTR 912) on digestive enzyme activity, growth performance, immune response, and disease resistance of black sharkminnow (*Labeo chrysophekadion*) against *Aeromonas hydrophila* infection. *Aquaculture Reports*, 27:101409.

Kusdarwati, R., Sudarno, & Hapsari, A. (2016). Isolation and indentification of fungi on the gold fish (*Carassius auratus*) in the fish market Gunung Sari Surabaya East Java. *Jurnal Ilmiah Perikanan dan Kelautan*, 8(1):1–15.

Leakey, R., Fuller, S., Treloar, T., Stevenson, L., Hunter, D., Nevenimo, T., Binifa, J., & Moxon, J. (2008). Characterization of tree-to-tree variation in morphological, nutritional and medicinal properties of *Canarium indicum* nuts. *Agroforestry Systems*, 73:77–87.

Lee, G. Y., & Han, S. N. (2018). The role of vitamin E in immunity. *Nutrients*, 10(11):1614.

Li, J., Zhou, L., & Woo, N. Y. S. (2003). Invasion route and pathogenic mechanisms of *Vibrio alginolyticus* to silver sea bream *Sparus sarba*. *Journal of Aquatic Animal Health*, 15(4):302–313.

Lim, K. C., Yusoff, F. M., Shariff, M., & Kamarudin, M. S. (2021). Dietary astaxanthin augments disease resistance of Asian seabass, *Lates calcarifer* (Bloch, 1790), against *Vibrio alginolyticus* infection. *Fish & Shellfish Immunology*, 114:90–101.

Lim, T. K. (2012). Edible medicinal and non-medicinal plants (1st Vol). Dordrecht: Springer.

Liu, H., Wang, Y., Cao, J., Jiang, H., Yao, J., Gong, G., Chen, X., Xu, W., & He, X. (2020). Antimicrobial activity and virulence attenuation of citral against the fish pathogen *Vibrio alginolyticus*. *Aquaculture*, 515:734578.

Liu, H. P., Wen, B., Chen, Z. Z., Gao, J. Z., Liu, Y., Zhang, Y. C., Wang, Z. X., & Peng, Y. (2019). Effects of dietary vitamin C and vitamin E on the growth, antioxidant defence and digestive enzyme activities of juvenile discus fish (*Symphysodon haraldi*). *Aquaculture Nutrition*, 25(1):176–183.

Liu, X., Steele, J. C., & Meng, X. Z. (2017). Usage, residue, and human health risk of antibiotics in Chinese aquaculture: A review. *Environmental Pollution*, 223:161–169.

Loo, K. Y., Letchumanan, V., Law, J. W. F., Pusparajah, P., Goh, B. H., Ab Mutalib, N. S., He, Y. W., & Lee, L. H. (2020). Incidence of antibiotic resistance in *Vibrio* spp. *Reviews in Aquaculture*, 12(4):2590–2608.

Lulijwa, R., Rupia, E. J., & Alfaro, A. C. (2020). Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers. *Reviews in Aquaculture*, 12(2):640–663.

Mailoa, M., Widyaningsih, T. D., Putri, W. D. R., & Harijono. (2019). Effect of walnut (*Canarium vulgare* L.) provisioning on white rat biology. *EurAsian Journal of BioSciences*, 13(1):213–217.

Mao, F., Liu, K., Wong, N. K., Zhang, X., Yi, W., Xiang, Z., Xiao, S., Yu, Z., & Zhang, Y. (2021). Virulence of *vibrio alginolyticus* accentuates apoptosis and immune rigor in the oyster *Crassostrea hongkongensis*. *Frontiers in Immunology*, 12:746017.

Mohamad, N., Amal, M. N. A., Yasin, I. S. M., Saad, M. Z., Nasruddin, N. S., Al-saari, N., Mino, S., & Sawabe, T. (2019a). Vibriosis in cultured marine fishes: a review. *Aquaculture*, 512:734289.

Mohamad, N., Roseli, F. A. M., Azmai, M. N. A., Saad, M. Z., Yasin, I. S. M., Zulkiply, N. A., & Nasruddin, N. S. (2019b). Natural concurrent infection of *Vibrio harveyi* and *V. alginolyticus* in cultured hybrid groupers in Malaysia. *Journal of Aquatic Animal Health*, 31(1):88–96.

Mohammadian, T., Monjezi, N., Peyghan, R., & Mohammadian, B. (2022). Effects of dietary

probiotic supplements on growth, digestive enzymes activity, intestinal histomorphology and innate immunity of common carp (*Cyprinus carpio*): a field study. *Aquaculture*, 549:737787.

Munaeni, W., Widanarni, Yuhana, M., Setiawati, M., & Wahyudi, A. T. (2020). Effect in white shrimp *Litopenaeus vannamei* of *Eleutherine bulbosa* (Mill.) Urb. Powder on immune genes expression and resistance against *Vibrio parahaemolyticus* infection. *Fish & Shellfish Immunology*, 102:218–227.

Nayak, S., Yogev, U., Kpordzaxor, Y., Zhu, Z., Gur, N., Gross, A., & Zilberg, D. (2023). From fish excretions to high-protein dietary ingredient: Feeding intensively cultured barramundi (*Lates calcarifer*) a diet containing microbial biomass (biofloc) from effluent of an aquaculture system. *Aquaculture*, 562:738780.

Pattah, H., Wahjuningrum, D., Yuhana, M., & Widanarni, W. (2021). Control of *Vibrio alginolyticus* infection in Asian seabass *Lates calcarifer* using Ambon banana plant powder *Musa paradisiacal* through the feed. *Indonesian Aquaculture Journal*, 15(2):85–91.

Ransangan, J., Lal, T. M., & Al-Harbi, A. H. (2012). Characterization and experimental infection of *Vibrio harveyi* isolated from diseased Asian seabass (*Lates calcarifer*). *Malaysian Journal of Microbiology*, 8(2):104–115.

Ransangan, J., & Mustafa, S. (2009). Identification of *Vibrio harveyi* isolated from diseased Asian seabass *Lates calcarifer* by use of 16S ribosomal DNA sequencing. *Journal of Aquatic Animal Health*, 21(3):150–155.

Rawling, M. D., Merrifield, D. L., Snellgrove, D. L., Kühlwein, H., Adams, A., & Davies, S. J. (2012). Haemato-immunological and growth response of mirror carp (*Cyprinus carpio*) fed a tropical earthworm meal in experimental diets. *Fish & Shellfish Immunology*, 32(6):1002–1007.

Rudrapal, M., Khairnar, S. J., Khan, J., Dukhyil, A. B., Ansari, M. A., Alomary, M. N., Alshabrmi, F. M., Palai, S., Deb, P. K., & Devi, R. (2022). Dietary polyphenols and their role in oxidative stress-induced human diseases: insights into protective effects, antioxidant potentials and mechanism (s) of action. *Frontiers in Pharmacology*, 13:283.

Sharma, S. R. K., Rathore, G., Verma, D. K., Sadhu, N., & Philipose, K. K. (2012). *Vibrio alginolyticus* infection in Asian seabass (*Lates calcarifer*, B loch) reared in open sea floating cages in India. *Aquaculture Research*, 44(1):86–92.

Silvaraj, S., Md Yasin, I. S., A. Karim, M. M., & Saad, M. Z. (2020). Elucidating the efficacy of vaccination against vibriosis in *Lates calcarifer* using two recombinant protein vaccines containing the outer membrane protein K (r-OmpK) of *Vibrio alginolyticus* and the DNA chaperone J (r-DnaJ) of *Vibrio harveyi*. *Vaccines*, 8(4):660.

Sokoudjou, J. B., Fodouop, S. P. C., Djoueudam, F. G., Kodjio, N., Kana, J. R., Fowa, A. B., Kamsu, G. T., & Gatsing, D. (2019). Antisalmonellal and antioxidant potential of hydroethanolic extract of *Canarium schweinfurthii* Engl. (Burseraceae) in *Salmonella enterica* serovar Typhimurium-infected chicks. *Asian Pacific Journal of Tropical Biomedicine*, 9(11):474.

Sumithra, T. G., Sharma, S. R. K., Neelima, L., Dhanutha, N. R., Joshy, A., Anusree, V. N., Gayathri, S., Raghu, R. K., Praveen, N. D., Thomas, S., & Rajesh, K. M. (2022). Red sea bream iridovirus infection in cage farmed Asian sea bass (*Lates calcarifer*): Insights into the pathology, epizootiology, and genetic diversity. *Aquaculture*, 548:737571.

Tadese, D. A., Song, C., Sun, C., Liu, B., Liu, B., Zhou, Q., Xu, P., Ge, X., Liu, M., Xu, X., Tamiru, M., Zhou, Z., Lakew, A., & Kevin, N. T. (2022). The role of currently used medicinal plants in aquaculture and their action mechanisms: A review. *Reviews in Aquaculture*, 14(2):816–847.

Talpur, A. D., & Ikhwanuddin, M. (2012). Dietary effects of garlic (*Allium sativum*) on haemato-immunological parameters, survival, growth, and disease resistance against *Vibrio harveyi* infection in Asian seabass, *Lates calcarifer* (Bloch). *Aquaculture,* 364-365:6–12.

Talpur, A. D., Ikhwanuddin, M., & Bolong, A. A. (2013).

Nutritional effects of ginger (*Zingiber officinale* Roscoe) on immune response of Asian sea bass, *Lates calcarifer* (Bloch) and disease resistance against *Vibrio harveyi*. *Aquaculture,* 400–401:46–52.

Turgay, E., & Karataş, S. (2016). First report of Vibrio harveyi infection in diseased common dentex (Dentex dentex) cultured in Turkey. *Süleyman Demirel Üniversitesi Eğirdir Su Ürünleri Fakültesi Dergisi*, 12(2):170–176.

Uribe-Querol, E., & Rosales, C. (2020). Phagocytosis: our current understanding of a universal biological process. *Frontiers in immunology*, 11:1066.

Van Hai, N. (2015). The use of medicinal plants as immunostimulants in aquaculture: A review. *Aquaculture*, 446:88–96.

Vinha, A. F., Moreira, J., & Barreira, S. V. P. (2013). Physicochemical parameters, phytochemical composition and antioxidant activity of the algarvian avocado (*Persea americana* Mill.). *Journal of Agricultural Science*, 5(12):100–109.

Wang, L., Ma, B., Chen, D., Lou, B., Zhan, W., Chen, R., Tan, P., Xu, D., Liu, F., & Xie, Q. (2019). Effect of dietary level of vitamin E on growth performance, antioxidant ability, and resistance to *Vibrio alginolyticus* challenge in yellow drum *Nibea albiflora*. *Aquaculture*, 507:119–125.

Wang, Q., Liu, Q., Ma, Y., Rui, H., & Zhang, Y. (2007). LuxO controls extracellular protease, haemolytic activities and siderophore production in fish pathogen *Vibrio alginolyticus*. *Journal of Applied Microbiology*, 103(5):1525–1534.

Wedemeyer, G. A., & Yasutake, W. T. (1977). Clinical methods for the assessment of the effects of environmental stress on fish health. Washington D.C.: U.S. Fish and Wildlife Service.

Wen, L., Lin, S., Zhu, Q., Wu, D., Jiang, Y., Zhao, M., Sun, J., Luo, D., Zeng, S., & Yang, B. (2013). Analysis of Chinese olive cultivars difference by the structural characteristics of oligosaccharides. *Food Analytical Methods*, 6:1529–1536.

Wibowo, C. H., Oktarina, E., Wardani, R. D., & Ngibad, K. (2019). The potential of walnut seeds (*Canarium indicum* L.) in repairing the genotoxicity effect of mercury on mice erythrocytes. *Borneo Journal Of Pharmacy*, 2(2):48–54.

Yang, R., Han, M., Fu, Z., Wang, Y., Zhao, W., Yu, G., & Ma, Z. (2020). Immune responses of Asian seabass *Lates calcarifer* to dietary *Glycyrrhiza uralensis*. *Animals*, 10(9):1629.

Yu, W., Yang, Y., Zhou, Q., Huang, X., Huang, Z., Li, T., Wu, Q., Zhou, C., Ma, Z., & Lin, H. (2022). Effects of dietary *Astragalus polysaccharides* on growth, health and resistance to *Vibrio harveyi* of *Lates calcarifer*. *International Journal of Biological Macromolecules*, 207:850–858.

Zhang, N. N., Guo, W. H., Hu, H., Zhou, A. R., Liu, Q. P., Zheng, B. D., & Zeng, S. X. (2018). Effect of a polyphenol-rich *Canarium album* extract on the composition of the gut microbiota of mice fed a high-fat diet. *Molecules*, 23(9):2188.

Zhou, Q., Wang, L., Wang, H., Xie, F., & Wang, T. (2012). Effect of dietary vitamin C on the growth performance and innate immunity of juvenile cobia (*Rachycentron canadum*). *Fish & Shellfish Immunology*, 32(6):969–975.