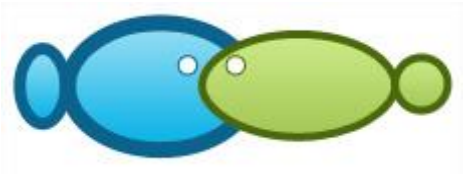

Effectiveness of extracts moringa leaves *(Moringa oleifera)* and Indian almond leaves *(Terminalia cattapa)* as immunostimulants in hybrid groupers *(Ephinephelus fuscoguttatus x E. lanceolatus)*

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Abstract. The hybrid grouper (*Ephinephelus fuscoguttatus* x *Ephinephelus lanceolatus*) has been successfully cultivated and has a high market value with high economic value. The purpose of this study was to determine the effectiveness of moringa (*Moringa oleifera*) leaf extract and Indian almond (*Terminalia catappa*) leaf extract in increasing the non-specific immunity of hybrid grouper. This research was carried out at the National Research and Innovation Agency's hatchery in Gondol, Bali, Indonesia, for 30 days of observation. The results demonstrated that the optimal survival and growth performance of hybrid grouper was obtained by giving Indian almond leaf extract. This treatment showed the highest number in survival (95.00%), specific growth rate (5.50% day^{-1}), and relative specific growth rate (116.64%) compared to the control group. The numbers of hematological parameters such as white blood cells, lymphocytes, red blood cells, hemoglobin concentration, hematocrit level, and platelet count showed that they varied, but tended not to differ significantly between treatments (P>0.05). The values of Total Plate Count and Total *Vibrio* sp. in this study had similar patterns, showed that they varied and there was no statistically significant difference (P>0.05). Overall, the findings indicate that Indian almond leaf extract can be employed as an immunostimulant in hybrid grouper fish.
Key Words: hybrid grouper, Moringa, Indian almond, immunostimulant, hematological.

Introduction. The hybrid grouper (*Epinephelus* sp.), formerly known as cantang grouper in Indonesia, is the result of crossbreeding between female tiger grouper (*Epinephelus fuscoguttatus* (Forsskål, 1775)) and male giant grouper (*Epinephelus lanceolatus* (Bloch, 1790)). This hybrid grouper has been successfully cultivated and has a high market value (Ismi et al 2012). The development of hybrid grouper farming is still limited by mortality restrictions, one of which is induced by infection with pathogenic microorganisms. *Vibrio* spp. are opportunistic pathogens in aquatic environments that frequently cause wounds or ulcers. *Vibrio anguillarum*, *V. harveyii*, *V. ordalii*, and *V. salmonicida* are bacteria that commonly infect and kill cultivated fish (Austin & Austin 2007). Natural components or medicinal plants are employed as alternative immunostimulants to improve the health of aquaculture products (Citarasu et al 2002; 2003). According to Punitha et al (2008) and Aderolu et al (2017), herbal medicines are efficient in treating infectious diseases without generating adverse effects like synthetic antibiotics. Fish immune systems and immunological responses can be strengthened by immunostimulants, which can be biological sources and synthetic sources (Pandey 2012). Numerous investigations have been carried out to identify plant-based bioactive compounds possessing antibacterial and antimicrobial characteristics, which could be employed to avert illnesses that affect aquaculture organisms (Abutbul et al 2005; Bamishaiye et al 2011; Farika et al 2014; Ahmed et al 2019). Various plants or their parts have been demonstrated to promote growth, boost immunity, reduce stress, and protect farmed fish from pathogenic microbes (Shakya 2017). Moringa leaves (*Moringa oleifera*) and Indian almond leaves (*Terminalia*

cattapa) are commonly found in tropical and subtropical areas. Moringa leaves are rich in nutrients and antioxidants. This plant is easy to cultivate and adapts to the environment, making it useful in aquaculture, animal husbandry, medicine, and agriculture (Abdel-Latif et al 2022). Moringa leaves also contain active substances such as kaempferol and isoquercitrin, that have antibacterial, hepatoprotective, anti-inflammatory, and hypolipidemic effects (Abd El-Gawad et al 2020; Nepolean et al 2009; Toppo et al 2015). Furthermore, this plant contains bioactive components such as flavonoids, steroids, alkaloids, phenolics, terpenoids, and essential oils (Arora et al 2013; Jung et al 2009).

Indian almond is a shade plant that thrives and is found throughout Southeast Asia (Tampemawa et al 2016). Indian almond leaves contain natural chemicals that can reduce the pH of water (Caruso et al 2013; Priyanto et al 2016; Bryan 2017). The tannin content of Indian almond leaves contributes to a decrease in water acidity (Ikhwanuddin et al 2014). Indian almond leaves also contain high levels of metabolites such as flavonoids, phenolic compounds, alkaloids, tannins, saponins, triterpenes, phytosterols, and steroidal glycosides (Cock 2015; Divya et al 2018). Indian almond leaves have been used to promote the growth and survival of fish farming, including betta fish (*Betta* sp.) (Waris et al 2018), gourami (*Osphronemus goramy* Lacepède, 1801) (Setiawan et al 2019), carp (*Cyprinus carpio* Linnaeus, 1758) (Aminah et al 2014), Nile tilapia (*Oreochromis niloticus* (Linnaeus, 1758)) (Priyanto et al 2016), cardinal tetra (*Paracheirodon axelrodi* (Schultz, 1956)) (Nurhidayat et al 2016), and black tiger shrimp (*Penaeus monodon* Fabricius, 1798) (Ikhwanuddin et al 2014). The purpose of this study was to determine the effectiveness of Indian almond leaf extract and moringa leaf extract in increasing the non-specific immunity of hybrid grouper (*E. fuscoguttatus* x *E. lanceolatus*).

Material and Method

Fish source. This study used hybrid groupers (*E. fuscoguttatus* x *E. lanceolatus*) from the National Research and Innovation Agency's hatchery in Gondol, Bali, Indonesia. For one week, 500 test fish were acclimatized in two 1,000-m^3 concrete tanks. Fifty fish were initially randomly sampled and measured the total length with a ruler and total weight with digital balances (LiBROR® EB-430S) that were the ranging in length from 4.0 to 6.5 cm and weight from 1.32 to 4.57 g. Before use, the fish were immersed in freshwater with 100 ppm formalin for 15 minutes with intense aeration to eliminate the risk of parasite infestation on the fish's gills and skin.

Extract herbal. Fresh moringa and Indian almond leaves taken from the tree are washed with running water and drained in a sieve. 50 grams of moringa and Indian almond leaves are weighed separately and blended with 100 mL of sterile distilled water until smooth. The fine grind is filtered through a tea strainer, and the resulting liquid is filtered through a rotifer sieve. A total of 200 mL of the extract is uniformly sprayed on 1 kg of commercial feed (Megami®, 3) and dried in an oven at 50°C for 24 hours. The control treatment feed consists of commercial Megami® feed that has been equally sprayed with sterile distilled water in the same volume as the herbal extract treatment. The dry feed is kept in the refrigerator (4°C) until it is utilized. Herbal extracts are prepared and mixed with feed every week.

Treatment. Each of the 220 hybrid grouper fish (*E. fuscoguttatus* x *E. lanceolatus*) was placed in four fiber tanks with a volume of 500 L (55 fish per tank) and a flowing water system. Each hybrid grouper fish in the four fiber tanks received herbal extracts of I moringa and Indian almond leaves, as well as purified water as a control. For four weeks, feed was administered ad libitum three times each day (morning, afternoon, and evening). Every day, fish waste was cleaned from the tank's bottom.

Challenge test. The challenge test was carried out after four weeks of herbal extract administration. Each tank per treatment yielded 50 test fish. The fish were kept in 5 plastic tanks with a volume of 100 L per treatment (10 fish per tank) with aeration and a stagnant water system. All fish received intraperitoneal injections of *V. harveyi* bacterium (2.1 x 10^7

cfu mL^{-1}) up to 0.1 mL each fish. For two weeks, mortality observations were conducted on four tanks, while one tank was utilized to collect blood and bacterial isolations.

Blood profile. Ten hybrid grouper fish (2-3 fish per tank) were collected in the second and fourth weeks following treatment. The fish had previously been anesthetized with clove oil in seawater (0.2 mL L^{-1}) until unconscious. The fish were then placed on a cloth drenched in seawater, with the heads covered to keep them wet. A tuberculin injection lubricated with heparin was used to draw blood from the caudal vein. A total of 0.2-0.3 mL of blood per fish was collected in a sterile Eppendorf tube (1.5 mL) and promptly evaluated on an Auto Hematology Analyzer (RT-7600 for veterinarians). Blood was also drawn from 10 fish per treatment three days following the challenge test with *V. harveyi*.

Bacterial isolation. Bacterial isolation was performed in the second and fourth weeks following herbal extract administration. Following blood collection, ten fish from each treatment were dissected, and the distal intestine contents were collected with a sterile Eppendorf (1.5 mL) by cutting it with sterile scissors. A total of 0.1 mg of distal intestine contents were dissolved in 0.9 mL of sterile saltwater, then homogenized and diluted to 10^{-2} to 10^{-4}. The distal intestine contents solution was grown on TSA (Tryptic Soy Agar) and TCBSA (Thiosulfate-Citrate-Bile Salt-Sucrose Agar) media. Bacterial cultures were cultivated in an incubator at 30°C for 24 hours. Meanwhile, bacteria were isolated from the distal intestines of 5 tests per treatment fish three days following the *V. harveyi* challenge test.

Experimental parameters. Water quality parameters such as salinity, DO, pH, and temperature were assessed weekly following herbal extract treatment. The ratio (%) of the total number of fish that survived the research compared to the initial number of fish is known as the survival rate, and it was determined using the formula given by Goddard (2012) as follows:

$$SR = (N_t \times N_0^{-1}) \times 100$$

Where:
SR - survival rate (%);
N_t - number of fish at the end of the rearing (individual);
N_0 - number of fish at the beginning of the rearing (individual).

The specific growth rate (SGR) was determined using the following method, which was suggested by Lugert et al (2014):

$$SGR = [(\ln W_2 - \ln W_1)/t] \times 100$$

Where:
SGR - specific growth rate (% day^{-1});
W1 - average weight of the fish at the beginning of the rearing (g);
W2 - average weight of the fish at the end of the rearing (g);
t - rearing duration.

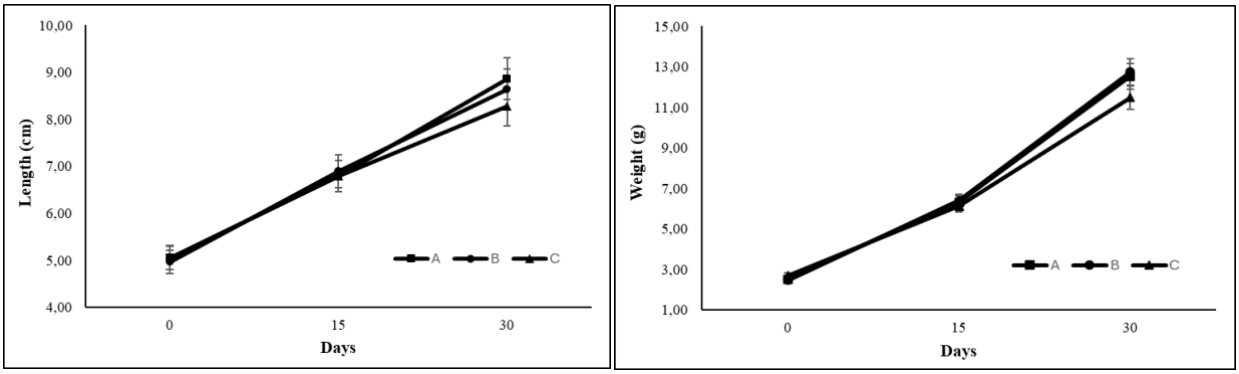
The relative specific growth rate (RSGR) was determined using the following method, which was suggested by Kusku et al (2018):

$$\text{Relative SGR} = \text{SGR} \times (100/\text{SGR of the control})$$

Where:
SGR - specific growth rate (% day^{-1}).

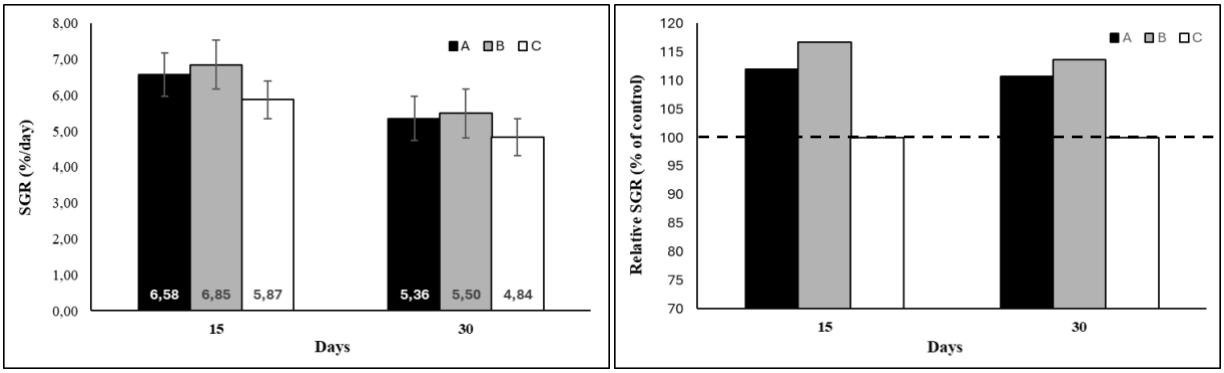
Statistical analysis. Analysis of variance (ANOVA) with a 95% confidence level was used to examine the survival rate, SGR, Relative SGR, length and weight growth, hematological parameters, and total bacteria. The Duncan Test was then used to determine whether the treatments had a significant effect.

Results. The results revealed that the average length of fish on the fifteenth day was very consistent across all three treatments. On the 30th day, treatment A had the largest average length (8.86±0.81 cm), followed by treatment B (8.64±0.50 cm) and treatment C (8.27±0.72 cm) (Figure 1a). Similarly, the average weight of fish across the three treatments followed the same pattern until the fifteenth day after treatment. After thirty days of treatment, treatment B had the highest average weight (12.74±1.75 g), followed by treatment A (12.53±2.85 cm) and treatment C (11.48±2.60 cm) (Figure 1b). Statistically, the increase in length and weight of fish during 30 days of treatment did not differ substantially (P>0.05) between treatments A, B, and C.



(a) (b)

Figure 1. Length (a) and weight (b) trend of hybrid grouper fish fed with different herbal extracts for 15 days and 30 days. Different letters show significant differences at 0.05 levels. Keys: A: Moringa leaves, B: Indian almond leaves, C: Control.



(a) (b)

Figure 2. SGR (a) and Relative SGR (b) trend of hybrid grouper fish fed with different herbal extracts for 15 days and 30 days. Bars with different letters show significant differences at 0.05 levels. Keys: A: Indian almond leaves, B: Moringa leaves, C: Control.

The SGR (specific growth rate) trend varied between days 15 and 30. On day 15, treatment B (6.85%) had the highest value, followed by treatment A (6.58%) and treatment C (5.87%). On day 30, treatment B (5.50%) had the highest value, followed by treatment A (5.36%) and treatment C (4.84%). However, the SGR trend did not differ substantially (P>0.05) between treatments (Figure 2a). Treatments A and B showed higher relative SGR values (112.01% and 116.64%, respectively) compared to the control (C; 100.00%) after

15 days. On day 30, treatments A and B had higher relative SGR values (110.73% and 113.63%, respectively) than the control (C; 100.00%) (Figure 2b).

The survival rate (SR) of fish throughout 30 days of treatments was highest in treatment B (95.00%), followed by treatment A (92.50%) and treatment C (90.00%) (Figure 3). The survival rates for the three treatments were not substantially different (P>0.05).

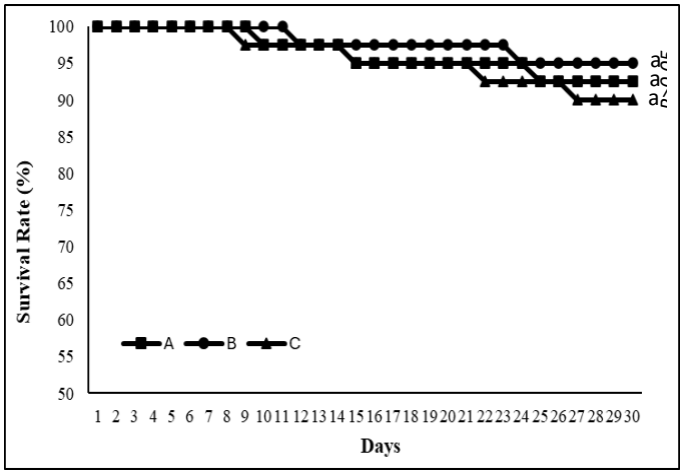


Figure 3. Survival Rate (%) of hybrid grouper fish fed with different herbal extracts. Other letters show significant differences at 0.05 levels. Keys: A: Moringa leaves, B: Indian almond leaves, C: Control.

Fish immunity. The results of observations of hematological parameters from the administration of moringa leaf extract (A) and Indian almond leaf extract (B) in feed until the 15th and 30th days are shown in Table 1. On the 15th day of observation, treatment A had the highest WBC, LYM, RBC, HGB, and HCT values, followed by C and B. However, the five parameters showed no significant difference between treatments (P>0.05). Treatment B had the highest PLT parameter value (P<0.05), followed by C and A. On the 30th day, LYM, RBC, HGB, and HCT values show a consistent pattern, with treatment A having the highest value followed by C and B. Treatment C shows higher WBC and PLT values than treatments B and A. The five parameters show no significant difference (P>0.05) between the three treatments, except for treatment A's HGB value, which differs significantly (P<0.05) from treatments B and C.

Table 1

Hematological parameters of hybrid grouper fish fed with different herbal extracts for 15 days and 30 days. Other letters show significant differences at 0.05 levels

Parameters	*Day 15*			*Day 30*		
	A	*B*	*C*	*A*	*B*	*C*
WBC (10^3 uL^{-1})	11.17±8.26^a	8.82±5.93^a	10.70±6.53^a	14.30±7.75^a	14.70±10.65^a	14.99±11.14^a
LYM (10^3 uL^{-1})	10.37±8.14^a	7.48±6.12^a	8.49±6.26^a	13.31±7.40^a	10.52±6.79^a	10.66±6.27^a
RBC (10^6 uL^{-1})	0.74±0.34^a	0.47±0.22^a	0.62±0.32^a	0.84±0.31^a	0.67±0.41^a	0.76±0.35^a
HGB (g dL^{-1})	9.10±2.36^a	7.55±2.15^a	8.98±1.63^a	9.30±1.12^a	7.72±1.72^b	7.77±1.32^b
HCT (%)	11.11±5.25^a	6.81±3.13^a	8.96±4.71^a	12.59±4.99^a	9.88±5.57^a	10.50±4.95^a
PLT (10^3 uL^{-1})	92.00±77.90^a	222.70±96.73^b	147.30±39.42^b	66.20±29.35^a	80.50±77.51^a	88.29±55.10^a

WBC: White blood cell (leukocyte) count, LYM: Lymphocytes, RBC: Red blood cell (erythrocyte) count, HGB: Haemoglobin concentration, HCT: Haematocrit level, PLT: Platelet count. The mean values in the same line with different superscripts are significantly different (P<0.05). Keys: A: Moringa leaves, B: Indian almond leaves, C: Control.

After 15 days of treatment, the total plate count (TPC) was seen to be higher in treatment B (6.97) than in treatments A (6.90) and C (6.79). The trend in TPC value was consistent until the 30th day after treatment, when treatment B had the highest number (6.84), followed by treatments A (6.83) and C (6.49) (Table 2). However, there was no significant difference (P>0.05) in TPC values on the 15th and 30th days between treatments. Following 15 days of therapy, treatment A's total Vibrio was found to be greater (5.20) than that of treatments B (5.11) and C (4.94). Before the 30th day following treatment, when treatment C had the highest number (5.67), followed by treatments A (4.97) and B (3.16) (Table 2). However, TPC values on the 15th and 30th days between treatments did not change significantly (P>0.05).

Table 2

Total Plate Count and Total Vibrio of hybrid grouper fish exposed to different herbal extracts for 15 days and 30 days. Other letters show significant differences at 0.05 levels

Treatments	*Total Plate Count (Log10)*		*Total Vibrio (Log10)*	
	Days 15	*Days 30*	*Days 15*	*Days 30*
A	6.90±0.31^{a}	6.83±0.09^{a}	5.20±1.34^{a}	4.97±1.18^{a}
B	6.97±0.39^{a}	6.84±0.82^{a}	5.11±1.92^{a}	3.16±1.26^{a}
C	6.79±0.11^{a}	6.49±0.13^{a}	4.94±1.17^{a}	5.67±0.59^{a}

The mean values in the same row with different superscripts are significantly different (P<0.05). Keys: A: Moringa leaves, B: Indian almond leaves, C: Control.

Challenge test. The challenge test with *V. harveyi* concentrations of 2.1 x 10^{7} cfu mL^{-1} resulted in low mortality or less pathogenicity. This was demonstrated by good survival rates and no significant difference (P>0.05) among the three treatments. Treatment B exhibited an SR value of 75%, which was slightly higher than treatments A (72.50%) and C (60%) (Figure 5).

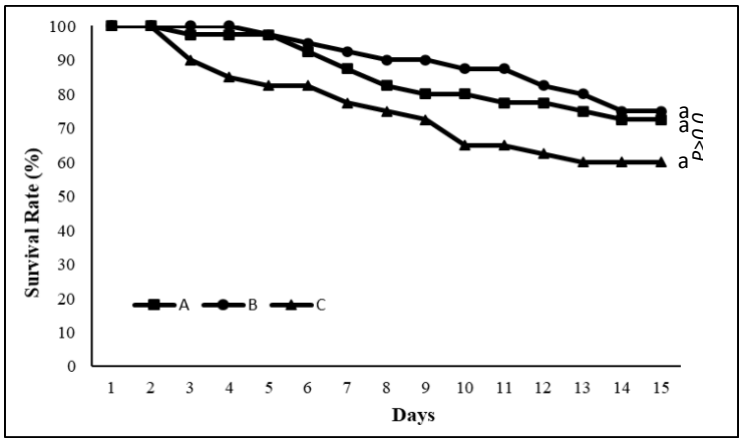


Figure 5. Survival Rate of hybrid grouper fish fed with different herbal extracts post bacterial challenge test. Other letters show significant differences at 0.05 levels. Keys: A: Moringa leaves, B: Indian almond leaves, C: Control.

Table 3

Total Plate Count and Total Vibrio of hybrid grouper fish exposed to different herbal extracts after challenge test. Other letters show significant differences at 0.05 levels

Treatments	*Total Plate Count (Log10)*	*Total Vibrio (Log10)*
A	8.63±0.46$^{a\ 8}$	5.05±0.70^{a}
B	8.70±0.84$^{a\ 8}$	5.08±0.83^{a}
C	8.18±0.24$^{a\ 8}$	6.41±0.56^{a}

The mean values in the same row with different superscripts are significantly different (P<0.05). Keys: A: Moringa leaves, B: Indian almond leaves, C: Control.

Total Plate Count (TPC) of the distal intestine contents in treatment B was slightly higher (8.70) than in treatments A (8.63) and C (8.18), but there was no statistically significant difference (P>0.05) between treatments; and treatment C's total Vibrio was found to be greater (6.41) than that of treatments B (5.08) and C (5.05) (Table 3).

The challenge test with *V. harveyi* resulted in higher WBC, LYM, HGB, and HCT values in treatment A compared to B and C. Treatment B yielded the highest RBC value, followed by A and C. Meanwhile, treatment C yielded the highest PLT result, followed by treatments A and B. The six parameters showed no significant difference between the three treatments (P>0.05) (Table 4).

Table 4

Hematological parameters of grouper fish fed with different herbal extracts post bacterial challenge test. Different letters show significant differences at 0.05 levels

Parameters	*Treatment*		
	A	*B*	*C*
WBC (10^3 uL^{-1})	16.12±11.75[a]	13.64±8.75[a]	14.39±7.93[a]
LYM (10^3 uL^{-1})	15.27±11.51[a]	12.62±8.38[a]	13.25±7.46[a]
RBC (10^6 uL^{-1})	0.96±0.41[a]	0.97±0.26[a]	0.84±0.27[a]
HGB (g dL^{-1})	8.56±2.49[a]	7.82±1.28[a]	8.19±1.42[a]
HCT (%)	14.11±5.69[a]	1410±3.60[a]	11.93±4.28[a]
PLT (10^3 uL^{-1})	54.10±36.89[a]	72.50±25.05[a]	90.90±62.52[a]

WBC: White blood cell (leukocyte) count, LYM: Lymphocytes, RBC: Red blood cell (erythrocyte) count, HGB: Haemoglobin concentration, HCT: Haematocrit level, PLT: Platelet count. The mean values in the same line with different superscripts are significantly different (P<0.05). Keys: A: Moringa leaves, B: Indian almond leaves, C: Control.

Discussion. The study results showed that adding moringa leaf extract and Indian almond leaf extract was proven to increase growth in length, weight, and survival rate. However, there was no significant difference in the results obtained. The growth in length and weight of hybrid grouper fish in this study also had a positive correlation. These results are also in line with the results of the study by Nugraha et al (2021) which explained that the addition of a dose of *T. catappa* leaf powder can increase survival rate and growth performance, and Saputra et al (2018) added that there was a correlation between length and weight which was linear after using *T. catappa* leaf powder treatment. The growth performance of *Megalobrama amblycephala* Yih, 1955 juveniles was not adversely affected by *T. catappa* supplementation in another investigation (Jiang et al 2023), which is in line with earlier research on guppy (*Poecilia reticulata* Peters, 1859) (Bisht et al 2020) and *O. niloticus* (Bbole et al 2016). These ingredients enhance fish health (Hoseinifar et al 2019a; Fazelan et al 2020) and can enhance fish growth performance by promoting assimilation and absorption and/or by suppressing energy expenditure brought on by stress triggers (Abdel-Tawwab 2016; Hoseinifar et al 2019b; Van Doan et al 2019), the use of herbal ingredients in aquaculture feed has become increasingly common in recent years.

The results of the study demonstrated that adding leaf extracts from Indian almonds and Moringa was proven to have a positive impact on growth parameters. This strengthens several previous studies that moringa leaves provide a significant effect and can increase growth rates (Ahmed et al 2014; Puycha et al 2017; Elabd et al 2019; Shourbela et al 2020; Tiimub et al 2020; Parveen et al 2021). Additionally, Francis et al (2001) noted that moringa is also the most effective source of protein, crude fiber, and fat. Lawal et al (2021), Ikhwanuddin et al (2014), and Ahmed et al (2005) have all demonstrated that *T. catappa* leaves also positively affect growth indices. This, according to Ji et al (2007), is because several medicinal plants improve fish growth performance by increasing the consumption of cellular lipids and fatty acids in addition to protein accumulation.

Hematological analysis is a commonly employed technique to acquire data regarding the physiology and overall health of fish. Blood is a complex mixture of many cell types, including platelets (analogs of mammalian platelets) (Lambert et al 2018), leukocytes (white blood cells), and erythrocytes (red blood cells) (Fazio et al 2019; Korytár et al 2013; Shen et al 2018). The hematological parameters (WBC, LYM, RBC, HGB, and

HCT) were measured on the fifteenth day of the study. The results showed that, except for one parameter, PLT (Platelet count) in treatment A, there were no significant differences. Results on the thirtyth day of the trial showed a similar trend, except for one parameter (HGB, or hemoglobin concentration) in treatment A that showed a significantly different value. Hematological parameters findings after the challenge test revealed variances in all treatments, although no significant difference (P>0.05) was seen in any of the parameters. The fact that hematological parameters did not significantly change could indicate that fish are not stressed when herbal extracts are added to their diet. Bahrami et al (2015) investigation yielded the same results that the lack of significant variation in hemoglobin content between diets may suggest that fish are not stressed by the use of herbal extracts as feed additives. This is because studies have indicated that stress can lead to a rise in the production of immature red blood cells from the head kidney as hematopoietic tissue, which can raise the content of hemoglobin in fish blood (Misra et al 2006). Furthermore, Nugroho et al (2017) found no discernible variation in the quantity of WBC, RBC, or Hb in *Betta* sp. treated with Ketapang leaf extract.

To provide oxygen and nutrients to every tissue in the fish's body, red blood cells are essential. Abnormal physiological processes in an organism, such as anemia, can be caused by a deficiency of red blood cells (Shen et al 2018). Erythrocytes, which make up the majority of blood cells, make sure that the various body tissues receive enough oxygen. A significant amount of erythrocytes are also drawn and mobilized from splenic depots due to metabolic changes brought on by physical exertion, excitement, and stress reactions, which raise the oxygen demand of tissues (Pearson & Stevens 1991). For example, after receiving roselle (*Hibiscus sabdariffa*) for two months as a dietary supplement, rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) considerably increased their blood's erythrocyte count while concurrently lowering their blood glucose and cortisol levels (Hoseini et al 2021). According to Wojtaszek et al (2002), unfavorable environmental circumstances might alter the makeup of circulating leukocytes as well as the quantity and form of erythrocytes. Fish blood leukocyte ratios can reveal information on how the fish will react to specific treatments or environmental factors, as has been observed in other vertebrates (Silva et al 2020). Seibel et al (2021) found that, generally, various stressors cause a decrease in lymphocyte numbers and an increase in myeloid cell mobilization in the bloodstream of impacted fish. White blood cell count is a measure of the body's defense mechanism in fish (Roberts 2012). Flavonoids from powdered *T. catappa* leaf increased fish immunity, which resulted in this elevated amount of white blood cells. Fish's non-specific immunity may be increased by flavonoids found in *T. catappa* leaves, which may act as biocatalysts in the synthesis of white blood cells (Nugroho et al 2016). Fish blood's hemoglobin parameter is a protein found in red blood cells. The fish get anemia due to low hemoglobin levels. Fish that dangle at the water's surface and move weakly are a sign of anemia. The blood's hemoglobin is responsible for carrying oxygen. Red blood cells' hemoglobin concentration determines their capacity to carry oxygen. High hemoglobin levels suggest that there is no metabolic issue in the fish because there is ample oxygen in the blood (Purnamawati et al 2017). Hematocrit is regarded as a primary measure of secondary stress response, along with hemoglobin (Hb) level and leukocyte count. Large, energetic fish typically have high oxygen demand in their muscles, which can induce erythropoiesis in the kidney's head (Jawad et al 2004). Accordingly, fish that reside in sedentary or bottom settings have a different physiological hematocrit than fish that swim quickly (Wells & Davie 1985). Abnormally low hematocrit readings are recognized as a telltale sign of anemia, a particular pathophysiological stress reaction. The electrolyte-water balance of fish blood may also affect hematocrit and Hb levels linked to erythrocyte count (Islam et al 2020).

The total plate count discovered in all treatments did not significantly differ (P>0.05), while the results varied on the 15th and 30th day of observation. Likewise, the total plate count after the challenge test had the same pattern, which did not show a significant difference (P>0.05). However, the addition of Moringa leaf extract and Indian almond leaf extract after the challenge test showed a higher total plate count than the control. The total Vibrio shows the same trend for all treatments. Indian almond was found to have a stronger antibacterial impact than Gram-negative strains of bacteria (*E. coli*,

Salmonella, *P. aeruginosa*, *V. parahaemolyticus*, and *A. caviae*) against Gram-positive bacteria (*S. aureus* and *E. faecalis*) in a prior study (Peixoto et al 2011). The components of moringa seed, such as pterygospermin, moringine, 4-(α-L-rhamnosyloxy)-phenyl acetonitrile glycosides, and 4-(α-L-rhamnosyloxy) benzyl isothiocyanate, prevent the growth of *Pseudomonas aeruginosa*, E*. coli*, *Shigella*, *and Streptococcus* (Jeon et al 2014). Strong antibacterial activity against *Pseudomonas aeruginosa*, *E. coli*, *S. aureus*, *Penicillium sclerotigenum*, and *Cladosporium cladosporioides* was demonstrated by Indian almond seeds (Oluduro et al 2010). The active ingredient in Indian almond leaves may be the cause of the bacterial inhibition, which Olusola et al 2013 and Nargis et al 2011 have confirmed that plant extracts have antibacterial properties. Thakur et al 2002 reported that extracts from *T. catappa* leaves have antibacterial properties against strains of *V. parahemolyticus* and *V. alginolyticus*. It was previously recognized that *V. parahaemolyticus* and *V. alginolyticus* were infectious agents in aquatic animals. Extracts from *T. catappa* leaves may be utilized as a readily available source of antibacterial agents to treat vibriosis in aquaculture (Nadirah et al 2013).

Conclusions. Based on the results of the study, the highest survival rate and the optimal growth performance of hybrid grouper fish (*E. fuscoguttatus* x *E. lanceolatus*) were obtained using Indian almond leaves extract (95.00%), with a specific growth rate of 5.50% day-1 and relative SGR 113.63% on day 30 of observation. The results after the challenge test also showed that the addition of Indian almond leaf extract had the highest SR of 75.00% and moringa leaves 72.50%, compared to the control of 60.00%. According to our research, hybrid grouper fish growth performance and survival rate can be improved by adding extracts from Indian almond leaves. Further research is needed regarding the optimal dosage for disease prevention in hybrid grouper fish.

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References

Abdel-Latif H. M. R., Abdel-Daim M. M., Shukry M., Nowosad J., Kucharczyk D., 2022 Benefits and applications of *Moringa oleifera* as a plant protein source in Aquafeed: A review. Aquaculture 547:737369.

Abdel-Tawwab M., 2016 Feed Supplementation to Freshwater Fish: Experimental Approaches. LAP LAMBERT Academic Publishing, Germany.

Abd El-Gawad E. A., El Asely A. M., Soror E. I., Abbass A. A., Austin B., 2020 Effect of dietary *Moringa oleifera* leaf on the immune response and control of *Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus*) fry. Aquac. Int. 28:389-402.

Abutbul S., Golan-Goldhirsh A., Barazani O., Ofir R., Zilberg D., 2005 Screening of desert plants for use against bacterial pathogens in fish. Israeli Journal of Aquaculture-Bamidgeh 7(2):71–80.

Aderolu A. Z., Lawal M. O., Soyinka O. O., Adeleke A. T., Bello M. D., 2017 Antimicrobial and antioxidant properties of African medicinal plants. Journal of Coastal Life Medicine 5(1):16-21.

Ahmed S. M., Swamy V., Dhanapal P. G. R., Chandrashekara V. M., 2005 Anti-diabeticactivity of *Terminalia catappa* Linn. leaf extracts in alloxan-induced diabetic rats. Iranian J Pharmacol & Therapeutics 4(1):36-39.

Ahmed H. S., Adel M., Adel E., 2014 Incorporation of *Moringa oleifera* leaf in Nile tilapia (*Oreochromis niloticus*) diet and its effect on growth performance and immune status. Journal of Veterinary Science 1(1):8.6-8.14.

Ahmed M., Ji M., Qin P., Gu Z., Liu Y., Sikandar A., Iqbal M. F., Javeed A., 2019 Phytochemical screening, totalphenolic and flavonoids contents and

antioxidanactivities of *Citrullus colocynthis* L. and *Cannabis sativa* L. Applied Ecology and Environmental Research 17(3):6961-6979.
Aminah, Prayitno S. B., Sarjito, 2014 The soaking effect of ketapang leaves (*Terminalia cattapa*) extract toward survival rate and histological liver of carp infected by *Aeromonas hydrophila*. Journal of Aquaculture Management Technology 3(4):118125.
Arora D. S., Onsare J. G., Kaur H., 2013 Bioprospecting of *Moringa* (Moringaceae): Microbiological Perspective. Journal of Pharmacognosy and Phytochemistry 1(6):193-215.
Austin B., Austin D. A., 2007 Bacterial Fish Pathogens. Diseases of Farmed and Wild Fish, 2nd Edition, Springer Praxis Publishing, Berlin.
Bahrami B. S., Paykan H. F., Dorafshan S., Mahboobi S. N., Vahabi M. R., 2015 Effect of dietary wood betony, *Stachys lavandulifolia* extracton on growth performance, haematological and biochemical parameters of common carp, *Cyprinus carpio*. Iranian J. Fisheries Science 14(4):805-817.
Bamishaiye E. I., Olayemi F. F., Awagu E. F., Bamshaiye O. M., 2011 Proximate and phytochemical composition of *Moringa oleifera* leaves at three stages of maturation. Advance Journal of Food Science and Technology 3(4):233-237.
Bbole I., Mumba C., Mupenda N., Kefi A. S., 2016 Analysis of growth performance and haematological parameters of *Oreochromis niloticus* fed on a varying diet of *Moringa oleifera* Lam. leaf meal as an additive protein source. International Journal of Fisheries and Aquaculture 8(11):105–111.
Bisht M., Kumar A., Shah T. K., 2020 Effect of *Moringa oleifera* leaf powder on skin mucosal immune responses and growth performance of guppy, *Poecilia reticulata* (Peter, 1860). Aquaculture Research 51:4984–4990.
Bryan M., 2017 *Terminalia catappa* (Talisay) leaves for preliminary surface water treatment: An eco-friendly approachs. Natural Products Chemistry and Research 5:1-4.
Caruso D., Lusiastuti A. M., Slembrouck J., Komarudin O., Legendre M., 2013 Traditional pharmacopeia in small scale freshwater fish farms in West Java, Indonesia: an ethnoveterinary approach. Aquaculture 416:334-345.
Citarasu T., Babu M. M, Sekar R. J. R., Marian P. M., 2002 Developing Artemia enriched herbal diet for producing quality larvae in *Penaeus monodon*, Fabricius. Asian Fish Sciences 15:21-32.
Citarasu T., Venket R. K., Sekar R. J. R., Babu M. M., Marian P. M., 2003 Influence of the antibacterial herbs, *Solanum trilobatum*, *Andrographis paniculata* and *Psoralea corylifoliaon* the survival, growth and bacterial load of *Penaeus monodon* post larvae. Aquaculture International 11:581-595.
Cock I. E., 2015 The medicinal properties and phytochemistry of plants of the genus *Terminalia* (Combretaceae). Inflammopharmacology 23:203-229.
Divya N., Rengarajan R.L., Radhakrishnan R., Fathi E., Allah A., Alqarawi A. A., Hashem A., Manikandan R., Vijaya A., 2018 Phytotherapeutic efficacy of the medicinal plant *Terminalia catappa* L. Saudi Journal of Biology Science 10–13.
Elabd H., Soror E., El-Asely A., El-Gawad E. A., Abbass A., 2019 Dietary supplementation of Moringa leaf meal for Nile tilapia *Oreochromis niloticus*: effect on growth and stress indices. The Egyptian Journal of Aquatic Research 45(3):265-271.
Farika E. Y., Suratma N. A., Damriyasa I. M., 2014 The Extract of *Moringa oleifera* Leaves as a Controller of *Argulus* sp Infestation on Comet Fish (*Carassius auratus auratus*). Veterinary Science and Medicine Journal 2(1):1-11.
Fazelan Z., Vatnikov Y. A., Kulikov E. V., Plushikov V. G., Yousefi M., 2020 Effects of dietary ginger (*Zingiber officinale*) administration on growth performance and stress, immunological, and antioxidant responses of common carp (*Cyprinus carpio*) reared under high stocking density. Aquaculture 518:734833.
Fazio F., Saoca C., Costa G., Zumbo A., Piccione G., Parrino V., 2019 Flow cytometry and automatic blood cell analysis in striped bass *Morone saxatilis* (Walbaum, 1792): a new hematological approach. Aquaculture 513:734398.

Francis G., Makkar H. P., Becker K., 2001 Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199(3-4):197-227.
Goddard S., 2012 Feed management in intensive aquaculture. Springer Science & Business Media, New York pp. 194.
Hoseini S. M., Hoseinifar S. H., Van Doan H., 2021 Growth performance and hematological and antioxidant characteristics of rainbow trout, *Oncorhynchus mykiss*, fed diets supplemented with Roselle, *Hibiscus sabdariffa*. Aquaculture 530:735827.
Hoseinifar S. H., Zou H. K., Van Doan H., Harikrishnan R., Yousefi M., Paknejad H., Ahmadifar E., 2019a Can dietary jujube (*Ziziphus jujuba* mill.) fruit extract alter cutaneous mucosal immunity, immune related genes expression in skin and growth performance of common carp (*Cyprinus carpio*). Fish Shellfish Immunol 94:705–710.
Hoseinifar S. H., Zou H. K., Paknejad H., Hajimoradloo A., Van Doan H., 2019b Effects of dietary white-button mushroom powder on mucosal immunity, antioxidant defence, and growth of common carp (*Cyprinus carpio*). Aquaculture 501:448–454.
Ikhwanuddin M., Moh J. H. Z., Hidayah M., Noor-hidayati A. B., Aina-Lyana N. M. A., Juneta A. S. N., 2014 Effect of Indian almond, *Terminalia catappa* leaves water extract on the survival rate and growth performance of black tiger shrimp *Penaeus monodon* post larvae. AACL Bioflux 7(2):85–93.
Islam M. J., Kunzmann A., Thiele R., Slater M. J., 2020 Effects of extreme ambient temperature in European seabass, *Dicentrarchus labrax* bream, acclimated at different salinities: growth performance, metabolic and molecular stress responses. Science of the Total Environment 735:139371.
Ismi S., Asih Y. N., Kusumawardani D., Prihadi T.H., 2012 [Grouper seed rearing as an effort to increase the income of coastal communities]. Prosiding Seminar Insentif Riset Sinas (INSINas2012). Bandung 29-30:312-318.
Jawad L. A., Al-Mukhtar M. A., Ahmed H. K., 2004 The relationship between haematocrit and some biological parameters of the Indian shad, *Tenualosa ilisha* (Family Clupeidae). Anim Biodivers Conserv 27:47–52.
Jeon S. R., Lee K. H., Shin D. H., Kwon S. S., Hwang J. S., 2014 Synergistic antimicrobial efficacy of mesoporous Zn loaded with 4-(α-L-rhamnosyloxy)-benzyl isothiocyanate isolatedfrom the *Moringa oleifera* seed. The Journal of General andApplied Microbiology 60(6):251-255.
Ji S. C., Takaoka O., Jeong G. S., Lee S. W., Ishimaru K., Seoka M., Takii K., 2007 Dietary medicinal herbs improve growth and some nonspecific immunity of red seabream *Pagrus major*. Fisheries Science 73:63-69.
Jiang W., Qian L., Zhao Y., Lin Y., Yang Y., Shen H., Ge X., Miao L., 2023 The Application of *Moringa oleifera* Leaf Meal and Its Fermentation Products in the Diet of *Megalobrama amblycephala* Juveniles. Fermentation 9:577.
Jung H. W., Tschaplinski T. J., Wang L., Glazebrook J., Greenberg J. T., 2009 Priming in systemic plant immunity. Science 324 (5923): 89-91. doi: 10.1126/science.1170025.
Korytár T., Thi H. D., Takizawa F., Köllner B., 2013 A multicolour flow cytometry identifying defined leukocyte subsets of rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunology 35(6):2017-2019.
Kusku H., Ergun S., Yilmaz S., Guroy B., Yigit M., 2018 Impacts of Urban Noise and Musical Stimuli on Growth Performance and Feed Utilization of Koi fish (*Cyprinus carpio*) in Recirculating Water Conditions. Turkish Journal of Fisheries and Aquatic Sciences 19(6):513-523.
Lambert F. N., Treberg J. R., Anderson W. G., Brandt C., Evans A. N., 2018 The physiological stress response of the Atlantic stingray (*Hypanus sabinus*) to aerial exposure. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 219–220:38–43.
Lawal M. O., Aderolu A. Z., Gafari W. A., 2021 Effects of *Terminalia catappa*, *Chromolaena odorata* and *Psidium guajava* Leaf Extracts on Growth, Biochemical and Haematology of *Clarias gariepinus*. FUW Trends in Science & Technology Journal 6(2):327–332.

Lugert V., Thaller G., Tetens J., Schulz C., Krieter J., 2014 A review on fish growth calculation: multiple functions in fish production and their specific application. Reviews in Aquaculture 8(1):30-42.
Misra C. K., Das B. K., Mukherjee S. C., 2006 The immunomodulatory effects of tuftsin on the nonspecific immune system of Indian Major carp, *Labeo rohita*. Fish and Shellfish Immunology 20(5):728-738.
Nadirah M., Wee T. L., Najiah M. 2013 Differential responses of *Vibrio* sp. to young and mature leaves extracts of *Terminalia catappa* L. International Food Research Journal 20(2):961-966.
Nargis A., Khatun M., Talukder D., 2011 Use of medicinal plants in the remedy of fish diseases. Bangladesh Research Publication Journal 5(3):192-195.
Nepolean P., Anitha J., Emilin R. R., 2009 Isolation, analysis and identification of phytochemicals of antimicrobial activity of *Moringa oleifera* Lam. Current Biotica 3:33–37.
Nugraha F. P. S., Rahardjo S., Saputra A., 2021 Survival and growth performance of snakehead juvenile (*Channa striata*) with various dosages of *Terminalia catappa* leaf powder. AACL Bioflux 14(2):762-773.
Nugroho R. A., Manurung H., Saraswati D., Ladyescha D., Nur F. M., 2016 The effects of *Terminalia catappa* L. leaves extract on the water quality properties, survival and blood profile of ornamental fish (*Betta* sp.) cultured. Biosaintifika: Journal of Biology & Biology Education 8(2):240-247.
Nugroho R. A., Manurung H., Nur F. M., Prahastika W., 2017 *Terminalia catappa* L. Extract improves survival, hematological profile and resistance to *Aeromonas hydrophila* in *Betta* sp. Arch Polish Fish 25:103–115.
Nurhidayat, Wardin L., Sitorus E., 2016 The survival and growth performance of juvenile cardinal tetra (*Paracheirodon axelrodi*) with application of tropical almond (*Terminalia catappa*) leaves. Nusantara Bioscience 8(1):1-4.
Oluduro O. A., Aderiye B. I., Connolly J. D., Akintayo E. T., Famurewa O., 2010 Characterization and antimicrobial activity of 4-(β-D-glucopyranosyl-1→ 4-α-L-rhamnopyranosyloxy)-benzyl thiocarboxamide; a novel bioactive compound from *Moringa oleifera* seed extract. Folia Microbiologica 55(5)422-426.
Olusola S. E., Emikpe B. O., Olaifa F. E., 2013 The potentials of medicinal plants extracts as bioantimicrobials in aquaculture. Int J Med Arom Plants 3(3): 404-412.
Pandey G., Madhuri S., Mandloi A. K., 2012 Medicinal plants useful in fish diseases. Plant Archives 12(1):1-4.
Parveen S., Rasool F., Akram M. N., Khan N., Ullah M., Mahmood S., Rabbani G., Manzoor K., 2021 Effect of *Moringa olifera* leaves on growth and gut microbiota of Nile tilapia (*Oreochromis niloticus*). Brazilian Journal of Biology 84:e250916.
Pearson M. P., Stevens E. D., 1991 Size and hematological impact of the splenic erythrocyte reservoir in rainbow trout, *Oncorhynchus mykiss*. Fish Physiology and Biochemistry 9:39–50.
Peixoto J. R. O., Silva G. C., Costa R. A., Fontenelle J. L. S., Vieira G. H. F., Filho A. A. F., Vieira R. H. S. F., 2011 In vitro antibacterial effect of aqueous and ethanolic *Moringa* leaf extracts. Asian Pacific Journal of Tropical Medicine 4(3)201-204.
Priyanto Y., Mulyana, Mumpuni F. S., 2016 Influence of almond leaf (*Terminalia catappa*) against growth and survival rate of Nile tilapia (*Oreochromis niloticus*) fry. Jurnal Pertanian 7(2):44-50.
Punitha S. M. J., Babu M. M., Sivaram V., Shankar V. S., Dhas S. A., Mahesh T. C., Immanuel G., Citarasu T., 2008 Immunostimulating influence of herbal biomedicines on nonspecific immunity in grouper *Epinephelus tauvina* juvenile against *Vibrio harveyi* infection. Aquaculture International 16:511–523.
Purnamawati, Djokosetiyanto D., Nirmala K., Surawidjaja E. H., Affandi R., 2017 Survival and growth responses of snakehead fish *Channa striata* Bloch. juvenile in aerated and unaerated acid sulfate water. Jurnal Akuakultur Indonesia 16(1):60-67.
Puycha K., Yuangsoi B., Charoenwattanasak S., Wongmaneeprateep S., Niamphithak P., Wiriyapattanasub P., 2017 Effect of moringa (*Moringa oleifera*) leaf supplementation

on growth performance and feed utilization of Bocourti's catfish (*Pangasius bocourti*). Agriculture and Natural Resources 51(4):286-291.
Roberts R. J., 2012 Fish pathology. Wiley-Blackwell, Oxford, UK, 590 p.
Saputra A., Ath-thar M. H. F., Samsudin R., Putri F. P., Prakoso V. A., 2018 Reproductive biology of striped snakehead (*Channa striata* Bloch, 1973) in Bogor and Bekasi, West Java. Berita Biologi 16(3):309-314.
Seibel H., Baßmann B., Rebl A., 2021 Blood Will Tell: What Hematological Analyses Can Reveal About Fish Welfare. Frontiers in Veterinary Science 8:616955.
Setiawan E. A., Susatyo N. A., Rahayu P., 2019 Effect of ketapang leaf extract (*Terminalia catappa*) on growth of gouramy fish (*Osphronemus gouramy* Lac.) in aquaculture systems. The Proceeding of the National Seminar on Science & Entrepreneurship VI, Semarang 7.
Shakya S. R., 2017 Effect of herbs and herbal products feed supplements on growth in fishes: A Review. Nepal Journal of Biotechnology 5(1):58-63.
Shen Y., Wang D., Zhao J., Chen X., 2018 Fish red blood cells express immune genes and responses. Aquaculture and Fisheries 3:14–21.
Shourbela R. M., El-Hawarry W. N., Am A. E. L., Abo-Kora S. Y., 2020 Potentiality of *Moringa oleifera* aqueous extract as a growth modulator and antistress in acute hypoxic Nile tilapia *Oreochromis niloticus*. Iranian Journal of Fisheries Science 1(1):67-84.
Silva M. B., Fraga R. E., Nishiyama P. B., da Silva I. S. S., Costa N. L. B., de Oliveira L. A. A., Rocha M. A, Junca F. A., 2020 Leukocyte profiles in *Odontophrynus carvalhoi* (Amphibia: Odontophrynidae) tadpoles exposed to organophosphate chlorpyrifos pesticides. Water, Air & Soil Pollution 231:372.
Tampemawa P. V., Pelealu J. J., Kandou F. E. F., 2016 [Test the effectiveness of ketapang leaf extract (*Terminalia catappa* L.) against bacteria *Bacillus amyloliquefaciens*]. Pharmacon-Jurnal Ilmiah Farmasi UNSRAT 5(1):308-320.
Thakur A. B., Vaidya B. D., Suryawanshi S. A., 2002 Pathogenicity and antibiotic susceptibility of *Vibrio* species isolated from moribund shrimps. Indian Journal of Marine Sciences 32:71-75.
Tiimub B. M., Mpanga I. K., Tiimob G. L., Tiimob R. W., Baani I., Tiimob E. L., 2020 Effect of *Moringa oleifera* feed supplements on all-male tilapia growth performance at Tano-Dumasi Pilot Aquaculture Centre. EAS Journal of Biotechnology Genetics 2(5):67-83.
Toppo R., Roy B. K., Gora R. H., Baxla S. L., Kumar P., 2015 Hepatoprotective activity of *Moringa oleifera* against cadmium toxicity in rats. Veterinary World 8:537–540.
Van Doan H., Hoseinifar S. H., Chitmanat C., Jaturasitha S., Paolucci M., Ashouri G., Dawood M. A., Esteban M. Á., 2019 The effects of Thai ginseng, *Boesenbergia rotunda* powder on mucosal and serum immunity, disease resistance, and growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings. Aquaculture 513:734388.
Waris A., Mansyur K., Rusaini R., 2018 Application of ketapang leaf powder (*Terminalia catappa*) with different doses and incubation temperatures against embryogenesisand hatching of Betta fish (*Betta splendens*) eggs. The Proceeding of the Nasional Symposium Marine and Fisheries V, Makasar, Indonesia pp. 24.
Wells R. M. G., Davie P. S., 1985 Oxygen binding by the blood and hematological effects of capture stress in two big gamefish: Mako shark and striped marlin. Comparative biochemistry and physiology. A, Comparative physiology 81:643–646.
Wojtaszek J., Dziewulska-Szwajkowska D., Łozinska-Gabska M., Adamowicz A., Dzugaj A., 2002 Hematological effects of high dose of cortisol on the carp (*Cyprinus carpio* L.): cortisol effect on the carp blood. General and Comparative Endocrinology 125(2):176–183.

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