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Grouper nursery development in sea floating net cage through the application of meniran and garlic powder in feed

Pengembangan pendederan ikan kerapu dalam KJA di laut dengan aplikasi tepung meniran-bawang putih dalam pakan

Irzal Effendi[1,2], Ahmad Trio Pratama[1], Aldy Mulyadin[3], Dinamella Wahjuningrum[1*]

[1]Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor, West Java, Indonesia
[2]Center for Coastal and Marine Resources Studies, IPB University, Bogor, West Java, Indonesia
[3]Department of Aquaculture, Pangkep State Polytechnic of Agriculture, Pangkep, South Sulawesi, Indonesia
*Corresponding author: dinamellawa@apps.ipb.ac.id

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ABSTRACT

An intensive nursery system in sea floating net cage (FNC) is being promoted to support the grouper farming, nowadays. An effort to maintain the fish health condition can be performed by applying phyto-pharmaceutical materials, such as *meniran* and garlic. This study aimed to evaluate the supplementation of *meniran*-garlic combination powder in feed on the health status and production performance of the grouper seed in sea FNC nursery. The grouper seed (8.27 ± 0.16 cm length and 10.89 ± 0.83 g weight) were reared in 1×1×1.5 m^3 FNC. The treatment feed was produced by supplementing 20 + 25 g/kg *meniran*-garlic powder through coating method. This study used three treatments, namely feeding the supplemented feed on the first 7 days (A) and 14 days (B) of maintenance, and feeding without the supplemented feed (control). Fish were reared for 42 days and sampled every 2 weeks to determine the fish health status, i.e. total erythrocytes, hematocrit level, hemoglobin level, total leucocyte count, and leukocyte differential. At the end of the study, growth performance containing survival rate, growth rate, specific growth rate, feed conversion ratio, and morphometric parameters were measured. The growth performance and fish health status obtained a significant increase ($P<0.05$) compared to the control. The supplementation of *meniran*-garlic powder in feed for 14 days improves the health status and growth performance of grouper.

Keywords: grouper, *meniran*-garlic, growth performance, health status

ABSTRAK

Sistem pendederan intensif dalam keramba jaring apung (KJA) di laut sedang digalakkan untuk mendukung usaha budidaya ikan kerapu. Salah satu upaya untuk menjaga kondisi ikan tetap sehat yakni pemberian fitofarmaka seperti meniran-bawang putih. Tujuan penelitian ini adalah mengevaluasi penambahan tepung meniran-bawang putih melalui pakan terhadap status kesehatan dan kinerja pertumbuhan benih ikan kerapu cantang pada pendederan dalam keramba jaring apung di laut. Benih ikan kerapu cantang (panjang 8.27 ± 0.16 cm dan bobot 10.89 ± 0.83 g) dipelihara dalam KJA berupa waring 1x1x1.5 m^3. Pakan perlakuan dibuat dengan penambahan tepung meniran-bawang putih 20 + 25 g/kg pakan dengan metode coating. Penelitian ini menggunakan tiga perlakuan yaitu pemberian pakan perlakuan selama 7 hari (A) dan 14 hari (B) pertama pemeliharaan, serta tanpa tepung meniran-bawang putih (kontrol). Ikan dipelihara selama 42 hari dan disampling setiap 2 minggu untuk dilakukan pengujian parameter kesehatan ikan yang terdiri dari jumlah eritrosit, kadar hematokrit, kadar hemoglobin, jumlah leukosit dan diferensial leukosit. Pada akhir penelitian dilakuan pengukuran kinerja pertumbuhan terdiri dari tingkat kelangsngan hidup, laju pertumbuhan, laju pertumbuhan spesifik, rasio konfersi pakan serta parameter morfometri. Hasil kinerja pertumbuhan dan status kesehatan ikan yang meningkat signifikan ($P<0.05$) dibandingkan dengan kontrol. Pemberian tepung meniran-bawang putih melalui pakan selama 14 hari dapat meningkatkan status kesehatan dan kinerja pertumbuhan ikan kerapu cantang.

Kata kunci: ikan kerapu cantang, meniran-bawang putih, kinerja pertumbuhan, status kesehatan.

INTRODUCTION

The grouper market demand and fishery processing product generally tends to increase although decreasing in the beginning of Covid-19 pandemic period (Trana *et al*., 2017; Love, 2021; Ribeiro *et al*., 2021). Indonesia becomes the main world's grouper exporter, and more than 90% of which is exported to Hongkong market at 165.87 tons on January, 2020 (Suhana, 2020). A hybrid grouper is a cross-bred grouper from female tiger grouper *Epinephelus fuscoguttatus* and male giant grouper *E. lanceolatus*. Hybrid grouper is a relatively fast-growing grouper (Fan *et al*., 2020) the present study examined the protective effect of dimethyl sulphoxide (Me_2SO; 8-12%, v/v. The main problem occurred in grouper culture business is seed availability in appropriate time, quality, quantity, and cost. Seeds originated from a hatchery is commonly obtained in a small size (4-9 cm), which will be harder if being reared in the marine grow-out system, mainly at the beginning of the rearing period. This condition causes a decreased survival rate and longer rearing period in the grow-out system (Effendi, 2019). To solve this problem, a nursery method should be performed (Effendi *et al*., 2021). Nursery is a business segmentation in aquaculture by growing the seeds from the hatchery to achieve greater size and stronger condition for growing-out business (Effendi, 2010).

Commonly, the grouper nursery at 4-9 cm size is carried out on land with concrete tank or fiber tank, before releasing to the sea at 9-11 cm or 11-13 cm (Ismi, 2010; Akbar *et al*., 2012; Ismi *et al*., 2012). The seed transportation cost in greater size will be relatively more expensive than in smaller size. Therefore, grouper seed nursery in a floating net cage (FNC) at sea is required as closed as possible to the growing-out production location unit. A nursery system at sea frequently faces several obstacles, such as high oceanographic dynamics and water quality compared to the nursery system on land which causes the cultured fish to be more potentially stressful and exposed to diseases (Hadiroseyani *et al*., 2010; Palm *et al*., 2011; Effendi *et al*., 2016; Dahlia *et al*., 2019; De *et al*., 2019). The economical impact of disease outbreaks is extremely significant as decreasing the grouper survival rate at 50-70% (Rimmer & Glamuzina, 2019). This condition requires a disease attack prevention on grouper nursery system.

The utilization of phytopharmaceuticals in culture activity as a prevention method has many been performed to support the sustainable aquaculture. Phytopharmaceuticals have various bioactivities, i.e anti-stress, immunostimulant, anti-microbes, and anti-parasites (Zhu, 2020; Dawood *et al*., 2021). Phytopharmaceuticals have several advantages compared to other prevention methods, namely having no side effect, non-dangerous material for the environment, and non-ecosystem disruptor material. Phytopharmaceuticals as alternative materials for preventing and recovering the cultured fish are relatively cheaper, more environmentally friendly, and more sustainable (Caruso *et al*., 2013). Garlic is one of the phytopharmaceutical plants that has long been utilized for parasite, fungi, bacteria, and virus infection medicines. Garlic is known to have an impact on the immune system and growth performance of the cultured fish, including cobia *Rachycentron canadum* and African catfish *Clarias gariepinus* (Lee & Gao, 2012; Guo *et al*., 2015; Gabriel *et al*., 2021).

In addition, *meniran* is also known to as herbs in traditional medications, mainly in a subtropical region. Several studies on phytochemicals reported that *menrian* contained many important compounds, such as polyphenols, flavonoids, triterpenes, and lignans (phyllanthin and hypophyllanthin), hydrolyzable tannins (ellagitannins), sterols, and alkaloids (Shanavas *et al*., 2019). Several previous studies have been identified that the *meniran* plant effect on aquatic animal immune response. Nhu *et al*. (2020) showed that the *meniran* plant extract supplementation in feed could induce the immune response of striped catfish *Pangasianodon hypophthalmus*. Vaname shrimp fed with *meniran* extract supplemented feed grew faster and was more resistant against the *Vibrio alginolyticus* infection (Ngo *et al*., 2020). This study aimed to evaluate the *meniran*-garlic powder supplementation in feed on the health status and growth of hybrid grouper in sea FNC nursery system.

MATERIALS AND METHODS

Meniran-garlic powder preparation

The *meniran*-garlic powder used in this study was obtained from the Biopharmacy Center of IPB University. The *meniran* powder was produced by washing the *meniran* bark, leaves, and roots with water until clean, before drying in the oven at 40°C for 18 hours. This sample was then grinded to become a powder with grinder

(Lestariningsih *et al*., 2015). The garlic powder production was produced by cutting the garlic thinly, before drying and grinding to become a powder using grinder.

Experimental design and feed treatment preparation

This study used a completely randomized design with three treatments and three replications. The treatments in this experiment contained *meniran*-garlic supplemented feed fed on the first 7 days of rearing period (A) and 14 days of rearing period (B), and feeding without the supplemented feed (control). The treatment feed was a commercial feed for grouper fish after supplementing with *meniran* and garlic powders at 20 + 25 g/kg feed (Wahjuningrum *et al*., 2016) through coating method. In this method, an egg and 100 mL water were used per 1 kg feed. The *meniran*-garlic powder, egg whites, and water were mixed and homogenized using a mixer. The homogenous material was mixed with the feed using a coating machine until evenly mixed. The mixed feed was dried in an oven at 50°C for 3-5 hours and stood for air-drying process.

Container and seed preparation

The grouper fish seed nursery was carried out in the Sea Farming Center, Center for Coastal and Marine Resources Studies, IPB University, *Semak Daun* Island, *Kepulauan Seribu*, Jakarta. In a 3×3×3 m^3 FNC, small net cages at 1×1×1.5 m^3 size were placed on the FNC with 0.5 cm mesh size. The 9 units of net cages were used in this study. The hybrid grouper seeds (8.27 ± 0.16 cm length and 10.89 ± 0.83 g weight) obtained from the Center for Brackishwater Culture Situbondo were reared for 28 days in FNC at Sea Farming Center. During adaptation, the hybrid grouper fish were fed three times a day, namely at morning, afternoon, and evening. Fish were sampled once a week soaked in a freshwater condition. After rearing for 28 days, fish were sampled and stocked in a 1×1×1.5 m^3 net cage.

Fish rearing

The grouper fish seeds at 100 fish/m^2 stocking density were reared for 42 days and fed with the feed treatment in apparent satiation three times a day at 08.00, 12.00, and 17.00 GMT+7, while the total feed intake was recorded. Every 2 weeks, the cage was replaced and fish were soaked in a freshwater every week for 2 minutes. The water quality parameters, namely temperature and dissolved oxygen were measured using a dissolved oxygen meter (DO meter) (*Lutron DO 5510*), while salinity level was measured with a refractometer (*Kenko*). Measurement was performed in the morning, afternoon, and evening at three-time frequency on a week.

Parameters

The fish health status parameters were observed on the 0^{th}, 14^{th}, 28^{th}, and 42^{nd} day of rearing period containing total erythrocytes (Blaxhall & Daisley, 1973), hemoglobin level (Wedemeyer & Yasutake, 1977), hematocrit level (Anderson & Siwicki, 1993), total leukocytes (Blaxhall & Daisley, 1973), and leukocyte differential (Amlacher, 1970). The production performance parameter measurement was performed at the 42^{nd} day of rearing period following Yan *et al*. (2016) which contained survival rate (SR), growth rate (GR), specific growth rate (SGR), and feed conversion ratio (FCR). The parameter formulas are presented below:

$$SR\ (\%) = N_t / N_0 \times 100$$

Note:
N_t was the final total fish at the end of rearing period (fish),
N_0 was the initial total fish at the beginning of rearing period.

$$GR\ (g/day) = (W_t - W_0)/t$$

$$SGR\ (\%/day) = 100\ (\ln W_t - \ln W_0)/t$$

Note:
W_t was the average of final fish weight at the end of rearing period (g)
W_0 was the average of initial fish weight at the beginning of rearing period (g),
t was the rearing period (days).

$$FCR = FI / (W_t - W_0).$$

Note:
FI was the total feed intake (g),
W_t was fish biomass at the end of rearing period (g),
W_0 was fish biomass at the beginning of rearing period (g).

The fish morphometry was measured at the end of rearing period after 42 days containing the final weight, total length, standard length, flesh length, and flesh height.

Data analysis

The data obtained were analyzed using an analysis of variance (ANOVA) at 95% confidence level using Microsoft Excel 2013 and *SPSS 22*. A significant different treatment was continuously analyzed using the Duncan's test.

RESULTS AND DISCUSSIONS

Results

Total erythrocytes, hematocrit level, and hemoglobin level

The measurement results of total erythrocytes obtained an increased value from the 0th day to 42nd day in all treatments. The measurent results of total erythrocytes at the 0th day was among 1.31–1.56 × 10^6 cells/mm^3. After the 14th day, increased total erythrocytes was occurred as the 7th day and 14th day obtained an insignificant different value (P>0.05) among the treatments, but having a significantly different from the control treatment (Figure 1A). Increased total erythrocytes was still occurred at 28th day and 42nd day, but showing a statistically insignificant different value among the treatments. The hematocrit level during the experimental period fluctuated extremely as the highest value was obtained on the 28th day (Figure 1B). The hematocrit value on the 0th day among the treatments were insignificantly different (P>0.05) at 37.11–38.95%. At the 14th day, the hematocrit level among the treatments were significantly different (P<0.05) with the highest value was obtained from the A treatment at 7th day, namely 43.98%. The hematocrit level value was insignificantly different among the treatments (P>0.05) at the 28th day and 42nd day. The measurement results of hemoglobin level during the rearing period increased in all treatments (Figure 1C). The hemoglobin level on the 0th, 14th, 28th, and 42nd day obtained a statistically insignificant different among the treatments (P>0.05).

Total leukocytes and leukocyte differential

The measurement results of total leukocytes increased from the 0th day to the 42nd day in all treatments (Figure 2A). The measurement results of total leukocytes on the 0th, 28th, and 42nd day

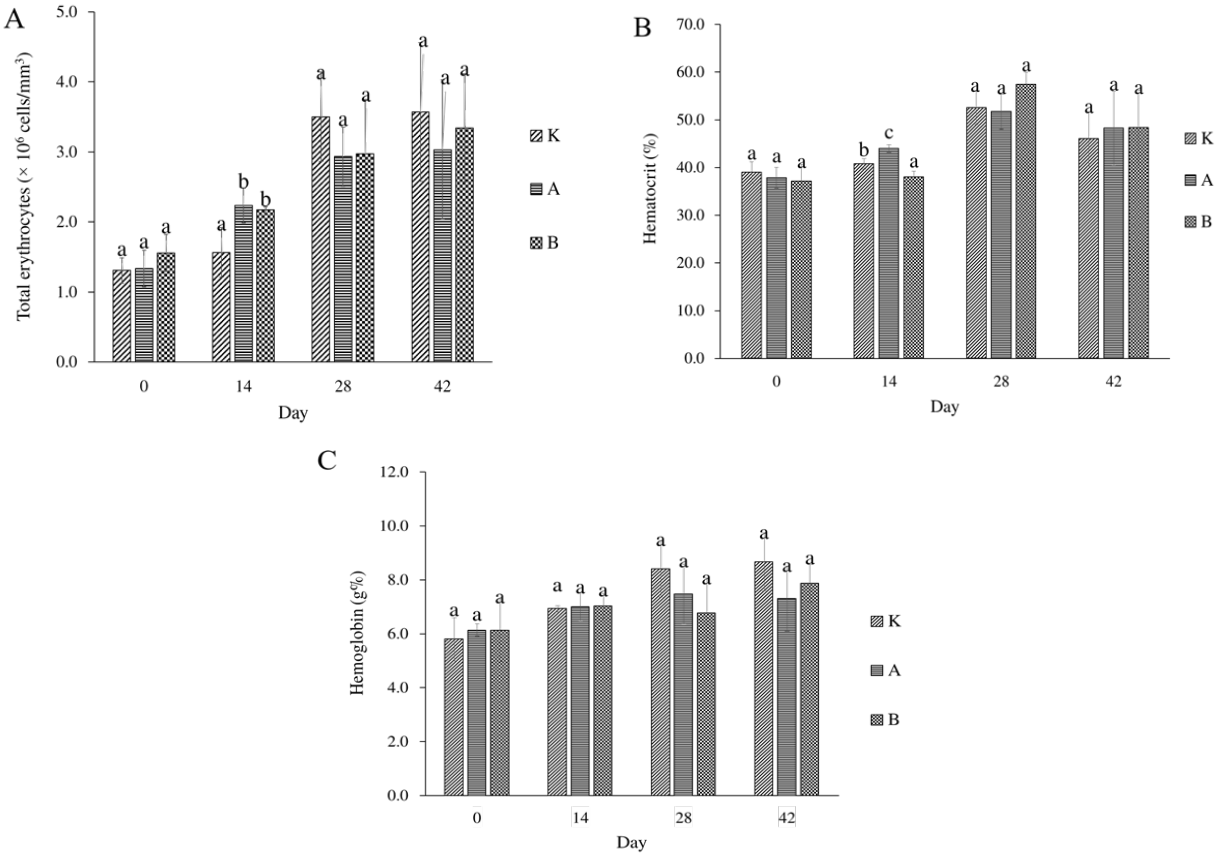

Figure 1. Total erythrocytes (A), hematocrit level (B), and hemoglobin level (C) of hybrid grouper *Epinephelus* sp. Different letters on each bar (mean ± standard deviation) show a statistical different value (Duncan's multiple range test; P<0.05). K was control treatment, A and B was *meniran*-garlic supplemented feed treatment fed on the first 7 and 14 days of treatment.

was insignificantly different (P>0.05) among the treatments. The total leukocytes on the 14th day was significantly different (P<0.05) among the treatments as the highest value was obtained from the 14th day (B), namely at 3.60 × 10^4 cells/mm^3. The leukocyte differential percentage value containing lymphocytes, monocytes, and neutrophils fluctuated during the rearing period. The measurement results showed that there was an insignificant difference (P>0.05) among the treatments on the 0th, 14th, 28th, and 42nd day. This condition is presented in Figure 2B.

Growth performance

The survival rate and specific growth rate values were insignificantly different among the treatments (Table 1). The growth rate of hybrid grouper after 14 days of feeding treatment period was higher (P<0.05) than the control treatment and 7 days of feeding treatment. The hybrid grouper fed with *meniran*-garlic powder supplemented feed both for 7 and 14 days obtained a better feed conversion ratio value than the control treatment (P<0.05).

Table 1. Production performance of hybrid grouper in nursery system fed with *meniran*-garlic powder supplemented feed after 42 days of rearing period

Parameters	Treatments		
	K	A	B
Survival rate (%)	93.33 ± 3.06[a]	94.33 ± 3.06[a]	94.67 ± 1.15[a]
Growth rate (g)	0.80 ± 0.02[a]	0.84 ± 0.04[a]	0.91 ± 0.01[b]
Specific growth rate (%/day)	3.27 ± 0.14[a]	3.38 ± 0.14[a]	3.79 ± 0.43[a]
Feed conversion ratio	0.95 ± 0.09[b]	0.79 ± 0.03[a]	0.79 ± 0.03[a]

[a]Values are presented as mean ± standard deviation. Different superscript letters in the same line show a significant different effect (P<0.05).

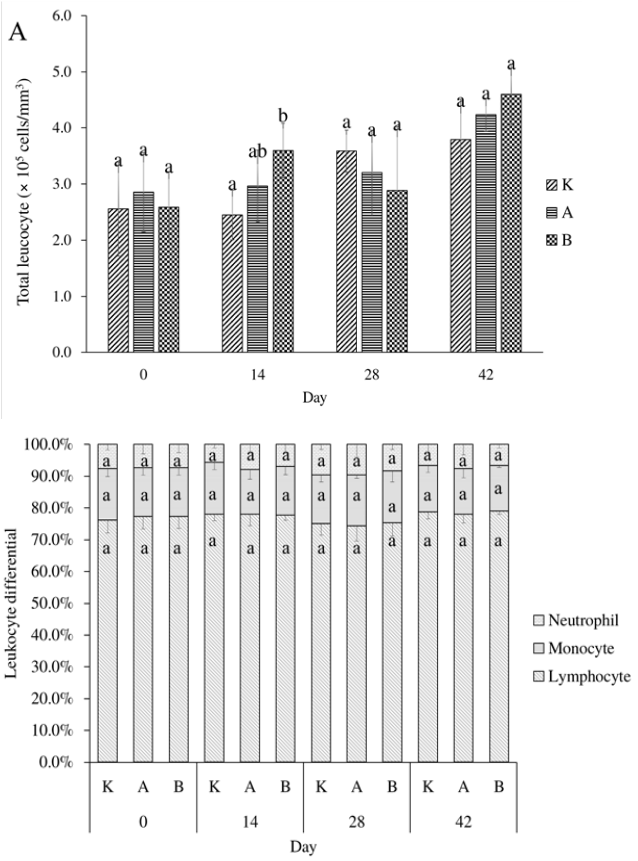

Figure 2. Total leukocytes (A) and leukocyte differential (B) of hybrid grouper *Epinephelus* sp. Different letters on each bar (mean ± standard deviation) show a statistical different value (Duncan's multiple range test; P<0.05). K was control treatment, A and B was *meniran*-garlic supplemented feed treatment fed on the first 7 and 14 days of treatment.

Fish morphometry

The morphometric measurement results of each treatment obtained a significant different result (P>0.05) as presented in Table 2. However, the 14-day feeding treatment had a higher morphometric value than the 7-day feeding and control treatments found in almost all parameters observed.

Water physico-chemical quality

The water quality measurent results during the rearing period obtained a temperature of 27–31°C, dissolved oxygenf (DO) of 4.2–9.5 mg/L, pH of 6.4–7.5, and salinity of 31–36 g/L. The water physico-chemical quality in FNC was still included in an optimum range for grouper fish nursery system.

Discussions

The fish health status improvement with plant extracts has been developed as preventive and treatment practices in a sustainable culture. Good preventive and treatment methods will not cause the occurrence of resistant pathogens and withdraw residues for the cultured animals (Dawood *et al*., 2021). Garlic and *meniran* plants release antioxidant and immunomodulator properties due to containing various biological compounds (Reverter *et al*., 2014). In this study, immunomodulatory effect in the combination of *meniran* and garlic powders was assessed based on the evaluation of blood hematological conditions, namely total erythrocytes, hematocrit level, hemoglobin level, total leukocytes, and leukocyte differential. Hematological profiles of cultured fish can commonly represent their physiological and health status (Fazio, 2019)"ISSN":"0044848 6","abstract":"Aquaculture is the fastest-growing food-production sector in the world. Aquaculture supplies 50% of all fish consumed globally today, and it is predicted that by 2030 it will be the prime source of fish. The use and validation of fish health monitoring tools have become increasingly evident due to the expansion of aquaculture. The complete blood cell count (CBC.

In this study, a better health status was found in hybrid grouper fish fed with the treatment feed (*meniran*-garlic supplementation) than in hybrid grouper fish fed without *meniran*-garlic supplemented feed (control). This condition was presented on the increased blood hematological profiles in fish fed with the treatment feed, mainly on 14 days of rearing period. This discovery was similar to the previous study that reported an increased health status of Nile tilapia after feeding with *meniran* and garlic supplemented feed (Fauziah *et al*., 2015). Erythrocyte is one of blood components functioned as an oxygen transporter. Erythrocytes carry O_2 to the tissue and release CO_2 and protons produced during the metabolism process (Sakuragui *et al*., 2019)R. fernandoi strain R28, which produces microcystin (MC. Increased erythrocyte volume was followed by more abundant oxygen availability which may result in a faster oxygen transfer (Witeska, 2013). Total erythrocytes of grouper fish seeds during the study increased and significantly affected the hemoglobin formation in blood to increase the oxygen binding activity (Samsisko *et al.*, 2014). This condition was similar to Gabriel *et al*. (2019)0.5, 1.0, 2.0, and 4.0%/kg diet, who mentioned an increased total erythrocytes of Africal catfish seed after feeding with garlic supplemented feed. In addition, Nile tilapia fed with *meniran* supplemented feed could also increase the total erythrocytes (A. Sivagurunathan *et al*., 2012).

High hematocrit value during the study indicates that the blood production is increasing. The total blood hematocrit level was different among the treatments. This condition was affected by two factors, namely environment and fish physiological condition (Setiawati *et al*., 2017). The hematocrit level during the study

Table 2. Morphometric parameters of hybrid grouper after the *meniran*-garlic powder supplementation feeding treatment and reared for 42 days.

Parameters	Treatments		
	K	A	B
Weight (g)	45.23 ± 1.76[a]	46.31 ± 1.72[a]	48.05 ± 2.63[a]
Total length (cm)	13.57± 0.15[a]	13.62 ± 0.12[a]	13.81 ± 0.25[a]
Standard length (cm)	11.88 ± 0.05[a]	11.87 ± 0.23[a]	12.03 ± 0.22[a]
Flesh length (cm)	7.79 ± 0.019[a]	7.91 ± 0.29[a]	8.09 ± 0.3-1[a]
Flesh height (cm)	3.89 ± 0.32[a]	3.66 ± 0.03[a]	3.72 ± 0.07[a]

[a]Numbers in the same column followed by the same letters have an insignificant different value (P>0.05)

was among 37-44%. This condition indicates that there are no negative effects after feeding the fish with *meniran* and garlic powder supplemented feed as fish did not suffer from anemia. Anemia occurs when the hematocrit level is less than 20% (Sakuragui *et al*., 2019). Meanwhile, hematocrit level greater than 45% is categorized as polycythemia (Grant, 2015)education, and aquaculture evolves. For the veterinary staff, fish handling, diagnostics, medicine, and surgery may require specialized training and equipment in comparison with terrestrial and arboreal animals, simply because of their aquatic nature and diversity. Fish hematology is one diagnostic tool that may not require additional equipment, may be inexpensive, and provide useful information in guiding treatment options. Challenges involving hematology may include handling and restraint, venipuncture, evaluation, and interpretation. In this article, strategies for these challenges are discussed for teleost (bony fish. Hemoglobin is an erythrocyte pigment functioned as an indicator to identify the number of bloods that can transport oxygen to the entire body (Witeska, 2013). The hemoglobin level during the study obtained an insignificant different value (P>0.05) among the treatments and was still in a normal range, namely .25–7.5 g%. Most teleostfish have hemoglobin level of 5–10 g% (Grant, 2015)education, and aquaculture evolves. For the veterinary staff, fish handling, diagnostics, medicine, and surgery may require specialized training and equipment in comparison with terrestrial and arboreal animals, simply because of their aquatic nature and diversity. Fish hematology is one diagnostic tool that may not require additional equipment, may be inexpensive, and provide useful information in guiding treatment options. Challenges involving hematology may include handling and restraint, venipuncture, evaluation, and interpretation. In this article, strategies for these challenges are discussed for teleost (bony fish. A lower hemoglobin level can cause a decreased oxygen intake (anoxia) (Burgos-Aceves *et al*., 2019).

Leukocyte is one of blood components functioned as a non-specific immune system. Leukocytes in blood are the most important cellular components in fish immune system to resist from bacteria and foreign material attacks (Lulijwa *et al*., 2019). In this study, the total leukocytes during the rearing period fluctuated as a significant different value was only occurred on the 14th day (P<0.05). Increased total leukocytes was a normal body reaction against foreign material infection that can alter the normal physiological process in fish (Suganthi *et al*., 2015). Phytochemicals contain various nutrients and chemical compounds as immune system stimulator, antimicrobial agent, and anti-stress agent for fish (Van Hai, 2015). The active components utilized as immune system stimulator comprise alkaloids, steroids, phenolates, tannins, terpenoids, saponnins, glycosides, and flavonoids (Awad & Awaad, 2017). The active compounds found in garlic and *meniran* that can induce the immune system are allicin (Lee & Gao, 2012), fructooligosaccharides (FOS) (Song *et al*., 2014), flavonoids, and tannins (Sarin *et al*., 2014)of which many are used as traditional medicines. The plant extracts have been used since ancient times, for treating hypertension, diabetes, hepatic, urinary, and sexual disorders, and other common ailments. Modern day scientific investigations have now confirmed pharmacognostic properties of Phyllanthus herbs. The phytochemicals attributing these medicinal properties have been identified in many of the Phyllanthus herbs. The morphologically similar herbs of Phyllanthus grow together and admixture of species during collection for manufacture of herbal medicines is quite common. Hence, along with pharmacognostic and phytochemical studies, appropriate protocols for correct identification of species are also important. As the use of these herbs as green medicines is becoming more popular, it is imperative to assess its genetic diversity and phylogenetic relatedness for future conservation strategies. This review is an attempt to present an overview of the existing studies on pharmacognostics, phytochemistry, species identification, and genetic diversity of Phyllanthus herbs and consequently (i.

The characteristics and total of leukocytes provides information about the fish health status as responsible in the immune response. Leukocytes have a big role in fish immune system containing lymphocytes (T-cell and B-cell) and phagocytes (monocytes and neutrophils) (Grant, 2015) education, and aquaculture evolves. For the veterinary staff, fish handling, diagnostics, medicine, and surgery may require specialized training and equipment in comparison with terrestrial and arboreal animals, simply because of their aquatic nature and diversity. Fish hematology is one diagnostic tool that may not require additional equipment, may be inexpensive, and provide useful information in guiding treatment options. Challenges involving

hematology may include handling and restraint, venipuncture, evaluation, and interpretation. In this article, strategies for these challenges are discussed for teleost (bony fish. The leukocyte differential containing lymphocytes, neutrophils, and monocytes obtained an insignificant different value among the treatments (P>0.05). Increased lymphocyte content in this study was because of the lymphocyte proliferation process as gaining a stimulus from garlic and *meniran* active compounds. The B-cell recognizes antigen species and produces an antibody (Song *et al*., 2014). Phagocyte cells (monocytes and neutrophils) in this study increased after treatment application. Phagocytosis in fish plays an important role in releasing pathogens (Palanikani *et al*., 2020) an opportunistic fish pathogen, which causes several major diseases including skin ulcer and haemorrhagic septicemia, contributes considerably to the lethality in aquaculture. Chemical and antibiotic treatment employed against *A. hydrophila* for disease management are expensive and consequently prompted the advent of drug resistance among the pathogens. To overcome these draw backs, alternative aquatic disease control methods using conventional plant-based medicines are focussed. Our present study aimed to augment the fish non-specific immune system with the implementation of methanolic crude extracts of *Andrographis paniculata* to Labeo rohita, for evaluating their efficacy against A. hydrophila. Histology of major organs of *A. hydrophila*-infected fish such as the gills and liver displayed severe tissue damage. *A. paniculata* extracts exhibited the strong antibacterial activity against *A. hydrophila* even at lower concentrations (50 µl.

Many studies prove that medicinal plants can be utilized as a feed attractant and growth promoter. In this study, growth rate and feed conversion ratio were significantly different from the control treatment (P<0.05). The biomedic herbal active compounds (flavonoids and polysaccharides) are identified as feed attractant that can promote the cultured fish growth (Pu *et al*., 2017). Garlic is known to be rich in polysaccharides. Polysaccharides are prebiotic materials or undigested feed materials that will stimulate the beneficial digestive tract microbiota growth and suppress pathogenic microbiota growth (Song *et al*., 2014). This condition has been proven by several studies in aquaculture. Based on Gabriel *et al*. (2019)0.5, 1.0, 2.0, and 4.0%/kg diet, polysaccharide extract in garlic could improve growth, feed utilization, and meat quality of African catfish *Clarias gariepinus*. The allicin content in garlic also had a positive effect on the intestine microflora to improve the digestion process (Moulia *et al*., 2018). *Meniran* is known to improve feed palatability and digestibility, resulting in a better fish growth and feed conversion level (Srivastava *et al*., 2019).

The specific growth rate in this study was insignificantly different among the treatments (P>0.05). The existence of anti-nutritional factors (ANFs) in herbs caused the feed digestibility level. ANFs are natural compounds in plants that can be consumed, but producing a negative effect in feed nutrient quality and toxic if being digested (Adeyemo & Onilude, 2013). Several ANFs that are abundant in plants are phytate, phenolic compounds (such as tannins), lectins, enzyme inhibitors (such as tripsin inhibitors), saponnins, and oxalate (Nikmaram *et al*., 2017). Treatments using garlic and *meniran* powder had ANFs which decreased the specific growth rate value. *Meniran* plant has tannins as part of ANFs (Narendra *et al*., 2012).

The survival rate of hybrid grouper during the rearing period was insignificantly different between commercial feed and treatment feed. This condition proves that the supplementation of garlic-*meniran* powder in feed can improve the fish health status that impacts on the hybrid grouper survival rate during the study. In culture activity, garlic and *meniran* are known as herbal materials to improve immune system and growth performance (Lee & Gao, 2012, Sunitha *et al*., 2017). The study results of Kustiana dan Suseno (2021) stated that *meniran* (*Phyllantus niruri*) and garlic (*Allium sativum*) could increase the survival rate of African catfish (*Clarias gariepinus*).

The morphometry parameters (final weight, total length, standard length, flesh length, and flesh width) in this study were insignificantly different (P>0.05) among the treatments. These study results indicate that the supplementation of *meniran*-garlic powder has no effect on fish body composition. The fish meal substitution with plant protein source produces a negative impact on the growth performance of carnivorous fish (Blaufuss & Trushenski, 2012). High tannins as ANFs in *meniran* produced a negative impact in nutrient digestibility rate which had no effect on the grouper fish morphometry. The negative effect of ANFs (tannins) are digestive disruption by binding the protein or minerals, causing the feed nutrient becomes hard to digest (Rathod & Annapure, 2016).

CONCLUSIONS

The supplementation of 20 + 25 g/kg *meniran*-garlic in feed on the hybrid grouper nursery system in sea FNC for 14 days is effective to improve the fish health status and growth performance.

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