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Effect of *Coleus blumei* Miana Leaf Extract in Feed on Whiteleg Shrimp Immune Response

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ABSTRACT *Coleus blumei* (miana) can be used as an antimicrobial, antibacterial, antioxidant, and anti-inflammatory. Research on miana leaf extract in feed to increase the immune response of whiteleg shrimp has not been conducted. This study aims to analyze the miana leaf extract in feed on the immune response of whiteleg shrimp. The study was conducted in July 2023 at the Hasanuddin University Laboratory and the Bogor Agricultural Institute Laboratory. The method was a completely randomized, four-treatment design with three replications: extracts of 0 g, 1 g, 2.5 g, and 7.5 g/kg of feed. The statistical results of the treatment gave a significant effect ($p<0.05$) on total hemocytes and phenoloxidase activity. Still, they did not have a significant impact ($p>0.05$) on the survival of whiteleg shrimp. The extract of 7.5 g/kg of feed had the highest impact on total hemocytes $8.43x10^3$ cells/mm^3, phenoloxidase activity 0.222 optical density λ=490, and 100% survival. The extract of 0 g/kg feed had the lowest impact on total hemocytes, 2.37 × 10^3 cells/mm^3, phenoloxidase activity of 0.09 Optical Density λ =490, and survival of 90%. Conclusion: *Coleus blumei* leaf extract in feed can improve the immune response and survival of whiteleg shrimp. The recommended concentration in aquaculture is 7.5 g/kg.

Keywords: *Coleus blumei*; phenoloxidase; survival; total haemocyte; whiteleg shrimp

INTRODUCTION

Whiteleg shrimp is one of Indonesia's leading fishery commodities that farmers widely cultivate. Cultivation of whiteleg shrimp is interesting to do because whiteleg shrimp has the characteristics of rapid growth, tolerance to a wide range of salinity (0.5-45 ppt), relatively low protein requirements of around 20-35%, low FCR values, and can be maintained at high stocking densities (Scabra *et al*., 2024). Whiteleg shrimp production in Indonesia has increased from year to year. In 2020, whiteleg shrimp production was known to be 881,601 tons, and increased by 941,647 tons in 2023 (KKP, 2024). Whiteleg shrimp, which was first introduced in Indonesia in 2001, now contributes more than 75 percent of Indonesia's total shrimp production (KKP, 2023). Production that increases from year to year reflects the community's need for cultivation results carried out on a semi-intensive and intensive scale to obtain high harvest yields (Soelistyoadi *et al*., 2019; Moreira *et al*., 2021; Kazangeldina *et al*., 2022).

Shrimp farming activities with the implementation of intensive systems have raised several problems, including a decrease in the carrying capacity of ponds for the life of the shrimp being farmed. Another impact is the emergence of a series of disease attacks limiting cultivation factors. Generally, crop failure occurs in shrimp farming due to attacks by bacterial or viral pathogens (Flegel, 2012; Sarjito *et al*., 2015; Ina-Salwany *et al*., 2019; Chen *et al*., 2025).

Whiteleg shrimp are organisms whose body defense system is non-specific against pathogen attacks, causing whiteleg shrimp to be more susceptible to disease (Lee & Shiau, 2004). The non-specific immune system is an innate immune system that functions as a first line of defense against various pathogens such as bacteria, viruses, fungi, and parasites, without recognizing the type of pathogen specifically. Because shrimp are invertebrates, they do not have an adaptive immune system like vertebrates (Tassanakajon *et al*., 2013; Li *et al*., 2021).

Shrimp diseases are divided into two groups, namely infectious diseases and non-infectious diseases. Infectious diseases are diseases caused by pathogenic organisms such as parasites, fungi, bacteria, and viruses that can be transmitted from one host to another through water, direct contact between hosts, intermediate hosts, equipment, and human activities. Non-infectious diseases are diseases caused by disorders that are not pathogenic, such as nutrition, water quality, toxins, and handling (Novriadi *et al*, 2014). When pathogens attack, the first to play a role in the shrimp defense system is the cuticle, which has antimicrobial properties through the mucus produced (Zheng *et al*., 2016). The next defense is hemocytes, which have an important role in the shrimp's internal defense system (Xian *et al*., 2021; Söderhäll *et al*., 2022).

Shrimp blood, called hemolymph, contains hemocyanin, which can function to transport oxygen throughout the body (Jungreis, 1980). Hemolymph also has immunoreactivity sensitive to the presence of foreign molecules, namely lectins. This lectin works actively because its specificity is strong and is formed from glucoproteins (Wang & Wang, 2013). Shrimp memory cells, when the pathogen invasion is complete, will produce lectins that will affect memory cells to lose their ability to remember infectious agents. Cells known as the primary defense of shrimp during pathogen invasion are hemocytes. Hemocytes can phagocytose foreign cells and wrap the foreign cells so that they are no longer active (Liu *et al*., 2020).

Several methods that have been carried out to control diseases in shrimp include the use of antibiotics, probiotics, vaccines, the use of specific pathogen-free

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(SPF), the implementation of biosecurity in cultivation areas, and the provision of immunostimulants, vitamins, and hormones. Antibiotics, as the most frequently used disease control method, have been banned because they can cause the emergence of antibiotic-resistant pathogens and contaminate cultivation areas (Pelic *et al*., 2024). An alternative to antibiotics is using natural ingredients to improve the immune system in shrimp.

One of the strategies for controlling shrimp disease is using natural ingredients, one of which is miana leaves, known for their various properties in treating various diseases. Miana is one of the plants included in the list of 66 biopharmaceutical plant commodities based on the Decree of the Minister of Agriculture Number: 511/Kpts/PD.310/9/2006 (Salimi, 2021). The bioactive compounds contained in miana leaves can function as antibacterials (Kusumawati *et al*., 2014; Basir *et al*., 2024), anti-inflammatories (Palette *et al*., 2017), immunomodulators (Pakadang *et al*., 2015), and antioxidants (Podungge *et al*., 2017). In vitro test of miana leaves (*Coleus scutellarioideas* (L) Benth) against *V. parahaemolitycus, Vibrio alginolyticus, and V. harveyii* bacteria showed that miana leaf extract (*Coleus scutellarioideas* (L) Benth) was able to inhibit the growth of vibrio bacteria with the highest inhibition in the chloroform fraction with an inhibition zone of 25 mm in *V. parahaemolitycus*, 26 mm in *V. alginolitycus*, and 20 mm in *V. harveyii* compared to the inhibition zone of oxytetracycline antibiotic 20 µg/ml, namely with an inhibition value of 25 mm for *V. parahaemolitycus*, 31 mm for *Vibrio alginolyticu—and*d 20 mm for *V. harveyii*. Therefore, purple miana leaves are considered capable of replacing the role of antibiotics in treating vibriosis (Basir *et al*., 2024). Also based on the results of research by Basir *et al*. (2025), through gas chromatography mass-spectrometry (GC-MS) tests, it was found that miana leaves of the *scutellarioides* (L) Benth type have active compounds that generally function as antibacterials, antivirals, anti-inflammatories, antioxidants and can increase total hemocytes, phagocytic activity, and survival of tiger shrimp through the addition of miana leaf extract to feed. This study aims to analyze the administration of extracts in feed on the immune response of whiteleg shrimp.

MATERIALS AND METHODS

Time and location of the research

This research was conducted in July 2023 at the Hatchery Laboratory, Faculty of Fisheries and Marine Sciences, Hasanuddin University. Miana leaf extraction was performed at the Basic Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Hasanuddin University. Analysis of bioactive compounds of miana leaves was carried out at the Center for Standardization and Service of Plantation, Mineral, Metal, and Maritime Industries. Observations of total hemocytes and phenoloxidase activity were performed at the Aquatic Organism Health Laboratory, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University.

Research materials

The research materials used were miana *Coleus blumei* leaves from Tana Toraja, South Sulawesi, 96% ethanol solvent brand Medika, specific pathogen free juveniles weighing 6 grams from the Takalar Brackish Water Aquaculture Center, feed adhesive boster progol, Grobest group brand whiteleg shrimp feed in the form of grower-sized pellets, sterile waterone aquades, whatman 45µ filter paper, Cheffy brand aluminum foil, Fulgid brand digital scales type gs lid 600gr x 0.01g 12 cm pan size, autoclave ican clave steam sterilizer STE 23 D class B, Fomac FGD-Z500 brand flour grinding machine, and IWAKI beaker glass volume 250-500 ml.

Research design

The study used an experimental pattern with a completely randomized design. It used mini *Coleus blumei* leaf extract at concentrations of 0, 1, 2.5, and 7.5 g/kg feed, with three replications each. The treatment used was a modification of Basir *et al*. (2023).

Preparation stage

This stage includes preparing tools and materials, flouring miana leaves, macerating miana leaves, preparing maintenance containers, testing animals, preparing feed, and maintaining. (A) Preparation of laboratory tools and media. Preparation is done by preparing all tools and materials to be used in sterile conditions. Tools and materials are sterilized in an autoclave using the wet sterilization method. Materials and tools to be sterilized must be safely wrapped by wrapping glassware with paper or other materials that do not damage the Pyrex material. The media sterilized in a tube or cup is also wrapped or closed neatly in an upright position so that the material does not spill or get damaged; (B) Extraction of Miana leaf simplicia. The procedure for preparing simplicia, according to Basir *et al*. (2023), is to collect miana plant samples, take the leaves, and then wash them clean. The samples are dried in the sun in the morning and evening by drying the samples on a tarpaulin and covering the top with a thin cloth. After drying, the samples are ground into flour using a grinding machine, then sieved, and are ready to be extracted. Extraction of miana leaf flour uses the maceration method by dissolving the sample using 96% ethanol with a sample to solvent ratio of 1:5 (w/v). Stirring is carried out for ± 10 minutes using a spatula and stored for 1 × 24 hours in a closed condition. The solution is filtered using filter paper to separate the filtrate from the dregs. The filtrate is collected in a container (Erlenmeyer), and the resulting dregs are soaked again with 96% ethanol. Soaking is done 3 times until the solution fades. The first, second, and third filtrates are mixed and evaporated until thick. The extract is stored in a closed glass container and is ready to use. If not yet used, the extract is stored in a refrigerator to maintain its stability (C)—preparation of containers and maintenance media.

The maintenance container is an aquarium measuring 30 cm x 40 cm x 35 cm. The aquarium is washed clean with fresh water and disinfected using chlorine at a dose of 30 µl/l for 24 hours. The aquarium is then rinsed with fresh water and dried. Furthermore, the outside of the aquarium is covered with black plastic, and the aquarium is filled with seawater with a volume of 35L, and aeration is installed. The water media have been filtered and sterilized from the Takalar Brackish Water Cultivation Center, with a salinity of

30 ppt. (D) Preparation of test animals. The test animals were specific pathogen-free juveniles weighing 6 grams from the Takalar Brackish Water Aquaculture Center. Before being used, whiteleg shrimp were adapted to the research environment for 3x24 hours at a salinity of 30 ppt with the salinity conditions of the original pond. During the acclimatization period, the shrimp were fed 3 times a day at satiation.

Furthermore, the shrimp were fasted for a day before being spread into the experimental container; (E) Preparation of test feed. The feed used is commercial feed that has nutritional content according to the growth needs of whiteleg shrimp. The test feed is made by mixing the dissolved extract into a liquid extract and spraying it evenly over the entire feed surface using a sprayer. The feed is sprayed with the extract while stirring until evenly distributed on all sides of the feed. The feed is then air-dried and resprayed with PEG adhesive dissolved in distilled water, at as much as 5 g/kg of feed. The feed is air-dried until dry, put into a dry and hygienic jar, and placed at room temperature, dry, and not humid.

Maintenance stage

Aeration was carried out before the test shrimp were spread into the maintenance container with a stocking density of 15 per aquarium. During the maintenance period, the shrimp were fed 3 times a day with a feed weight of 5 % of the shrimp biomass weight. Before each feeding, suction was carried out, and the water media were replaced with as much water as was lost due to suction. Daily water quality measurements were carried out for pH, temperature, salinity, DO, and ammonia parameters. Growth sampling was carried out once a week to determine individual weight gain and determine the feed dose for the following week.

Measurement of the immune system

The shrimp immune parameters measured consisted of phenoloxidase activity (PO) and total hemocytes (THC), measured based on Chiu *et al*. (2007) and Van Hai & Fotedar (2009).

Measurement of total hemocytes (THC)

The procedure for measuring total hemocytes refers to the method of Ekawati *et al*. (2012), namely, total hemocytes are observed by taking 0.1 ml of shrimp hemolymph from the base of the fifth walking leg using a syringe containing 0.2 ml of anticoagulant. The anticoagulant used is a mixture of 0.44 g of trisodium citrate, 0.99 g of sodium chloride, 0.19 g of EDTA, and 50 ml of distilled water. The mixture of hemolymph and anticoagulant is homogenized for 5 minutes. The first drop is discarded, and the second is dripped into the hemocytometer. Hemocytes were observed and counted using a microscope with 40x magnification. The observation data were entered into a formula to determine the total number of hemocytes.

$$\text{Total Hemocytes (cells/mL)} = \frac{\text{Number of cells counted}}{\text{Number of fields of view}} \times 10^4 \times DF$$

Caption:

DF = Dilution factor

Measurement of phenoloxidase (PO) activity

Measurement of phenoloxidase activity refers to the method of Ekasari *et al*. (2016), namely, PO activity is measured based on the formation of dopachrome produced by L-DOPA. A total of 1 ml of hemolymph-anticoagulant mixture was centrifuged at 1,500 rpm for 10 minutes at 4 °C. The supernatant was discarded, and the pellet was slowly resuspended in 1 ml of cacodylate-citrate buffer solution (0.01 M sodium cacodylate, 0.45 M sodium chloride, 0.10 M trisodium citrate, pH 7) and centrifuged again at the same speed. The pellet was then suspended in 200 µl of cacodylate-citrate buffer (0.01 M sodium cacodylate, 0.45 M sodium chloride, 0.10 M trisodium citrate, pH 7). A 100 µl cell suspension was then incubated with 50 µl trypsin (1 mg/ml cacodylate buffer) as an activator for ten minutes at a temperature of 25-26 °C. Then, 50 µL of L-DOPA (3 mg/ml of cacodylate buffer) was added after five minutes, and 800 µl of cacodylate buffer was added. Optical density (OD) was measured using a spectrophotometer at a wavelength of 490 nm. A standard solution containing 100 µl of hemocyte suspension, 50 µl of cacodylate buffer (a substitute for trypsin), and 50 µl of L-DOPA was used to measure the background PO activity in all test solutions. The optical density of PO activity under all test conditions was expressed as dopachrome formation in 50 µl of hemolymph.

Measurement of survival rate

The survival rate is measured by counting the number of test shrimp at the beginning and end of the study, then entering the following formula (Haliman & Adijaya, 2005).

$$SR = \frac{N_t}{N_o} \times 100\ \%$$

Caption:

SR Survival Rate (%)

N_t = Number of individuals at the end of the experiment (g)

N_o = Number of individuals at the start of the experiment (g)

Data analysis

The data obtained were analyzed using one-way ANOVA (Analysis of Variance) to determine the effect of treatment on the number of hemocytes, phenoloxidase activity, and the survival rate of whiteleg shrimp at a 95% confidence level. If the treatment has a significant effect, the W-Tukey HSD difference test is continued to determine the differences between treatments.

RESULTS AND DISCUSSION

Results of the gas chromatography-mass spectrometry (GC-MS) test

The results of the analysis of the gas chromatography-mass spectrometry (GC-MS) test of the chromatogram of 96% ethanol extract of miana leaves of the blumei type are presented in Figure 1.

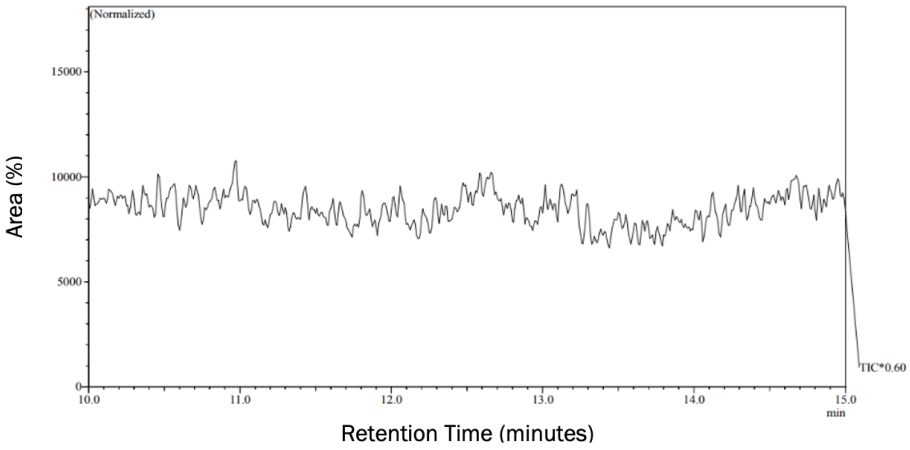

Figure 1. Chromatogram of *Coleus blumei* miana leaf extract.

Based on the results of the GC-MS test, *Coleus blumei* leaf extract contains 455 types of bioactive compounds with varying retention levels. Some compounds that have a significant retention time include, N-(glycylalanine) this compound appears at a retention time of 32,049 with an area concentration of 1.03%, 1-(4-Propylphenyl)-2-(4-pentoxyphenyl) ethyne appears at a retention time of 16,447 with an area concentration of 0.90%, N-Carbethoxy-N-methyl-2(3',4'-dimethoxy-1'-ph this compound appears at a retention time of 22,669 with an area concentration of 0.86%, cyclopropane, 1-bromo-1,2-dichloro-(CAS) 1-B appears at a retention time of 18,192 with an area concentration of 0.75%, 2-Amino-3-methylpentane this compound appears at a retention time of 21,341 with an area concentration of 0.73%, 9-methyl-1,2,3,9A-tetrahydrocarba appears at a retention time of 0.75 %, retention 0.75 %, 14,558 with area concentration 0.71 %, 2-Heptanamine, 6-methyl- (CAS) 2-Amino-6-m this compound appears at retention time 31.133 with area concentration 0.69 %, 1,2-diethyldiborane-2-B10 appears at retention time 20.906 with area concentration 0.67 %, Butanal (CAS) n-Butanal this compound appears at retention time 22.269 with area concentration 0.61 %, and DL-alanyl-DL-alanine this compound appears at retention time 29.975 with area concentration 0.60 %. The molecular structures of some of these compounds are presented in Table 1.

Table 1. Molecular structure of *Coleus blumei* miana leaf extract.

No	Compound Name	Area (%)	Classified as Compounds	Molecular Structure
1	N-(Glycyl)alanine	1.03	Flavonoid, alkaloid, saponin	$C_5H_{10}N_2O_3$
2	1-(4-Propylphenyl)-2-(4-pentoxyphenyl) ethyne	0.90	Flavonoid, saponin, alkaloid	$C_{22}H_{26}O$
3	N-Carbethoxy-N-methyl-2(3',4'-dimethoxy-1'-ph	0.86	Saponin, tanin	$C_{22}H_{25}N\,O_4$
4	Cyclopropane, 1-bromo-1,2-dichloro-(CAS) 1-B	0.75	Flavonoid	$C_3H_3BR\,CL_2$
5	2-Amino-3-methylpentane	0.73	Terponoid	$C_6H_{15}N$
6	9-MethyL-1,2,3,9A-Tetrahydrocarba	0.71	Protein	$C_{17}H_{11}N_5$
7	2-Heptanamine, 6-methyl- (CAS) 2-Amino-6-m	0.69	Terponoid	$C_8H_{19}N$
8	1,2-Diethyldiborane-2-B10	0.67	Alkaloid	$C_4H_{14\,10}B_2$
9	Butanal (CAS) n-Butanal	0.61	Aldehid, keton	C_4H_8O
10	DL-Alanyl-DL-Alanin	0.60	Amino	$C_6H_{12}N_2O_3$

The largest bioactive compounds contained in *Coleus blumei* miana leaf extract include N-(Glycyl) alanine, with a molecular structure of $C_5H_{10}N_2O_3$, included in amino acid compounds, which are basic and can be classified as flavonoid, alkaloid, and saponin compounds. Flavonoid compounds function as potent antioxidants that can ward off free radicals in the fish body, and improve fish growth performance (Ponomarev *et al*., 2021), improve the immune system (Nurkartika *et al*., 2023), and are antibacterial and antiviral. Alkaloid compounds can be helpful as antibacterials, antiparasitics, and immunomodulators. Meanwhile, saponin can function as an antiparasitic, a fat digestive, and an immunostimulant. Compound 1-(4-Profilphenyl)-2-(4-pentoxyphenyl) ethine, included in pantothenic acid compounds or commonly known as vitamin B complex, plays a role in the process of blood cell formation, and is basic, can be classified as an alkaloid, flavonoid, and saponin compound. Compound N-Carbetoxy-N-methyl-2(3',4'-dimethoxy-1'-ph with a molecular structure of $C_22H_25NO_4$, is a compound containing antibiotics that are bacteriostatic and bactericidal. Bacteriostatic properties can inhibit the growth of organisms but do not kill them. In contrast, bactericidal properties are antibiotics that kill bacteria or viruses. This compound can be classified as a saponin and tannin compound.

Other compounds such as Cyclopropane, 1-bromo-1,2-dichloro-(CAS) 1-B with the molecular structure $C_3H_3BRCl_2$, are halogenated organic compounds consisting of propane with bromine substituents on the methyl group, this compound contains anticancer substances, can be used as an intermediate for the synthesis of organic compounds, including agrochemicals and pharmaceutical drugs, can be classified as flavonoid compounds. Compound 9-methyl-1,2,3,9A-tetrahydrocarba, with the molecular structure $C_{17}H_{11}N_5$, is a compound that can inhibit aromatase to reduce the production of estrogenic steroid hormones. Aromatases, also called estrogen synthetase or estrogen synthase, are enzymes responsible for an important step in estrogen biosynthesis.CYP19A1 encodes this enzyme, a member of the cytochrome P450 group, which is a monooxygenase. Monooxygenase is an enzyme that combines one hydroxyl group into a substrate through various metabolic pathways. Compound 1,2-Diethyldiborane-2-B10 with molecular structure $C_4H_{14}10B_2$ is a compound of the quinone alkaloid group, an active compound in the cinchona plant. Butanal (CAS) n-Butanal with molecular structure C_4H_8O is a group of aldehyde and ketone compounds. Aldehydes and ketones are polar molecules due to the presence of double bonds C = O In addition to the presence of dispersion forces, there will also be an attractive force between permanent dipoles in adjacent molecules. Compound DL-Alanyl-DL-alanine with molecular structure $C_6H_{12}N_2O_3$ is a derivative of amino compounds. This compound plays a role in growth, immunity, and metabolism. In addition, this compound can also play a role in the wound healing process (Farrar & Krosnick, 1991).

Total hemocytes (THC)

Figure 2 presents data from observations of total hemocytes of white leg shrimp fed with the addition of Coleus blumei damiana leaf extract.

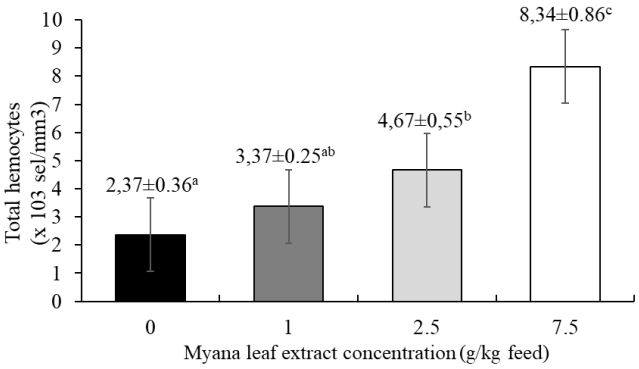

Figure 2. Number of hemocytes of whiteleg shrimp using feed with additional *Coleus blumei* miana leaf extract. The different superscript letters indicate significant differences (P<0.05) by Tukey's HSD.

The results of the statistical analysis of the variance test showed that the treatment of adding miana leaf extract, *Coleus blumei,* to the feed significantly increased the total hemocytes of whiteleg shrimp. The treatment that gave the highest effect was the addition of feed extract of 7.5 g/kg, which was different from other treatments. The increase in total hemocytes in Figure 1 aligns with the increase in *Coleus blumei* leaf extract concentration in the feed. The increase in total hemocytes of whiteleg shrimp in the treatment of adding miana leaf extract showed that miana leaf extract mixed with feed increased the immune response in whiteleg shrimp. According to Braak (2002), an increase in the total number of hemocytes is considered one form of cellular immunity response in the shrimp body. This shows that Miana leaf extract can stimulate the formation of hemocyte cells, so that hemocyte production in the whiteleg shrimp body increases.

The ability of miana leaves of the blumei type as an immunostimulant is thought to be due to the content of bioactive flavonoid compounds in miana leaves, which have properties that are readily soluble in water and function as antimicrobials, antiviruses, and immunostimulants (Naiborhu, 2002). According to Subagiyo (2009), the content of polyphenol compounds also has high antioxidant activity. In addition, Miana *Coleus blumei* leaves have also been known to contain DL-alanyl-DL-alanine compounds through gas chromatography mass index (GC-MS) tests, which play a role in immunity and metabolism. Several phytochemical compounds that can act as immunostimulants in *Coleus blumei* leaves are phenols, flavonoids, and methanol (Suparmanan & Saptarini, 2019).

Phenoloxidase activity

Figure 3 shows the results of observations of phenoloxidase activity, which shows differences in activity between

treatments.

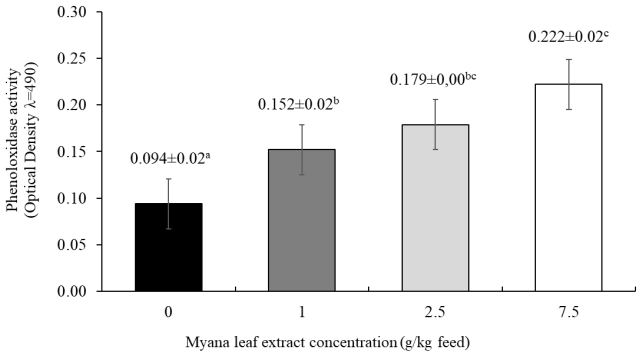

Figure 3. Phenoloxidase activity of whiteleg shrimp using feed with additional *Coleus blumei* miana leaf extract. The different superscript letters indicate significant differences (P<0.05) by Tukey's HSD.

The results of the analysis of variance showed that the addition of miana leaf extract, *Coleus blumei,* with various concentrations had a significant effect (p<0.05) on the phenoloxidase activity (enzyme) of whiteleg shrimp. The W-Tukey HSD further test results showed that adding miana leaf extract to the feed differed from the treatment without miana leaf extract to the feed or the 0 g/kg treatment. The highest phenoloxidase activity was found in the treatment of adding 7.5 g/kg of feed extract.

The addition of *Coleus blumei* leaf extract can increase phenoloxidase activity. This shows that *Coleus blumei* leaf extract can stimulate white leg shrimp hemocytes so that phenoloxidase activity is formed. Phenoloxidase activity increases the ability of white leg shrimp to recognize foreign particles entering the body, and then the phagocytosis process occurs. This process will reduce foreign particles entering the body so that the shrimp's immune system can increase (Pebrianto & Sukenda, 2010).

The phenoloxidase enzyme is produced through the prophenoloxidase system, which can be activated due to the presence of immunostimulants and enzymes that play a role in the melanization process. Adding blumei extract as an immunostimulant to the feed can increase phenoloxidase activity. This shows that blumei extract can stimulate whiteleg shrimp hemocytes so that phenoloxidase activity is formed. With increased phenoloxidase activity of whiteleg shrimp, the ability to recognize foreign particles entering the body is better, so phagocytosis can occur. This process will reduce foreign particles entering the body so that the shrimp's immune system can increase (Pebrianto & Sukenda, 2010).

According to Yeth *et al*. (2005), the activity of the phenoloxidase enzyme in whiteleg shrimp will increase after administration of immunostimulants. This increase is likely due to an increase in the activity of the protease enzyme, which is an activator of the formation of the phenoloxidase enzyme. Bahgat *et al.* (2002) in their study reported that the sensitivity of the protease and phenoloxidase enzymes in Biomphalaria glabrata hemocytes will increase the activity of the serine protease enzyme, followed by an increase in the activity of the phenoloxidase enzyme.

Survival rate

The survival rate of whiteleg shrimp at the end of the study can be seen in Figure 4.

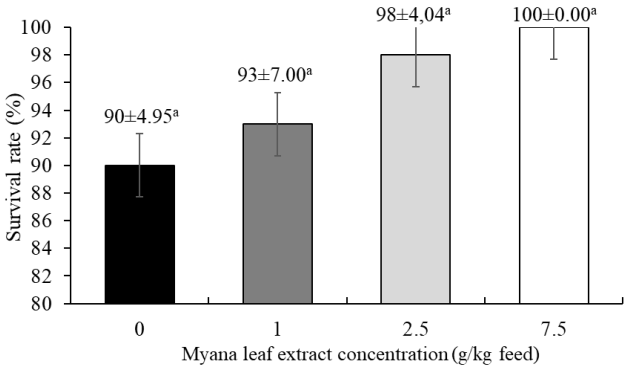

Figure 4. Survival rate of whiteleg shrimp with additional feed of Coleus blumei leaf extract. The different superscript letters indicate significant differences (P<0.05) by Tukey's HSD.

Based on the results of the variance analysis, it was shown that the addition of *Coleus blumei* miana leaf extract did not have a significant effect (p>0.05) on the survival of whiteleg shrimp during the maintenance period (Figure 4). Adding 7.5 g/kg *Coleus blumei* leaf extract found the highest survival rate, but it was not significantly different from other concentration treatments. This is because the commercial feed used has a composition and nutritional value that is not much different between treatments, and cano supports optimal shrimp survival with a survival value of 90-100%. However, no research has examined the addition of *Coleus blumei* leaf extract to whiteleg shrimp feed.

Similar studies using natural additives have shown that adding these additives can increase shrimp survival. However, the differences are often not statistically significant. For example, research by Rachmawati *et al*. (2020) showed that adding the amino acid lysine to commercial feed did not significantly affect the survival of wader shrimp. However, there was an increase in other parameters. Research by Prama *et al*. (2023) showed that using two different brands of commercial feed did not provide a significant difference in the survival of wader shrimp, with survival rates of 86% and 84%, respectively. This shows that the commercial feed used has a composition and nutritional value good enough to support optimal shrimp survival. In addition, research by Fahrudin *et al.* (2022) showed that variations in protein levels in commercial feed did not significantly affect the survival of juvenile whiteleg shrimp, which remained high in all treatments. This indicates that the commercial feed used is sufficient to meet the nutritional needs of

shrimp to achieve high survival.

The addition of *Coleus blumei* leaf extract at different concentrations between treatments also did not affect or change the nutritional value of the feed. Although the treatment did not affect the survival rate of white leg shrimp statistically, descriptively, we can see that the increase in survival value increased along with the increase in the concentration of purple mini leaf extract in the feed.

CONCLUSION AND RECOMMENDATION

Conclusion

This study concludes that adding Miana *Coleus blumei* leaf extract to the feed can increase the immune response of white leg shrimp. The extract is effective at a 7.5 g/kg feed concentration.

Recommendation

The leaves of *Coleus blumei* are recommended to be incorporated into shrimp feed as a natural additive with the potential to enhance shrimp's immune response. Its application in aquaculture systems is expected to strengthen shrimp's immune defense mechanisms, supporting their health and survival throughout the cultivation period.

AUTHORS' CONTRIBUTIONS

BB discovered the research idea, and BB, NLR, FA, and SRK developed and designed the experiment. UJ collected data and handled nearly all of the technical work. All authors contributed significantly to this publication, including data processing, analysis, and test preparation.

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