Supplementation of hairy eggplant (*Solanum ferox*) and bitter ginger (*Zingiber zerumbet*) extracts as phytobiotic agents on whiteleg shrimp (*Litopenaeus vannamei*)

Sinung Rahardjo¹, Merary A. The Vauza², Djumbuh Rukmono¹, Putu Angga Wiradana³

¹Department of Aquaculture, Faculty of Utilization Fisheries, Jakarta Fisheries University, Jakarta, Indonesia
²Master Student of Fisheries Resource Utilization, Postgraduate Program of Jakarta Fisheries University, Jakarta, Indonesia
³Study Program of Biology, Faculty of Health, Science, and Technology, Universitas Dhyana Pura, Provinsi Bali, Indonesia

**ABSTRACT**

**Objective:** This study aimed to evaluate the combination of hairy eggplant (*Solanum ferox*) and bitter ginger (*Zingiber zerumbet*) on the production performance and hematological parameters of whiteleg shrimp (*Litopenaeus vannamei*).

**Materials and Methods:** Four treatments were formulated in the test feed, where P1 (control + commercial vitamin C); P2 (120 ml/l combination of *S. ferox* and *Z. zerumbet*); P3 (100 ml/l); and P4 (80 ml/l) 6,000 post-larvae shrimp with an average initial weight of 0.2 gm were randomly stocked in four groups, with three replications per treatment, and 500 were stocked in each pond with a total of 12 pounds.

**Results:** Based on the results, there were significant differences in production performance (survival, absolute weight growth, specific growth rate, and feed conversion ratio). Biologically, the best performance was found in the P3 treatment (100 ml/l). In this treatment, the total number of hemocytes and the number of hyaline hemocytes were much higher, and this was not the case in the control treatment (P1), where the number of semi-granular and granular cells was significantly higher than the treatment group (*p < 0.05*).

**Conclusions:** This study confirmed that supplementation of 100 ml/l of *S. ferox* and *Z. zerumbet* could improve the production performance and hematological parameters of whiteleg shrimp, with functional potential to be developed in phytobiotic-based commercial diets for shrimp.

**Introduction**

Crustacean production has increased to 6.09 million tons, or equivalent to USD 36.2 million, due to increased consumption of shrimp commodities worldwide [1]. Whiteleg shrimp (*Litopenaeus vannamei*) are one of the most popular aquaculture commodities and are relatively easy to cultivate [2]. This can be proven by the high percentage of Pacific whiteleg shrimp cultivation worldwide, at around 90%. This shrimp can adapt to a broad salinity range (euryhaline) between 5 and 30 parts per trillion; cultivate with a high stocking density; and grow sufficiently with low protein feeds, making this shrimp a leading commodity in Indonesia.

However, an increase in shrimp production with unmatched water quality management, quality seeds, or superior broodstock to quality feed can harm shrimp productivity by causing various diseases. Several disease outbreaks in shrimp ponds are related to viral diseases like the white spot syndrome virus and Taura syndrome virus. On the contrary, bacterial diseases like *Vibrio harveyi*, *V. alginolyticus*, and *V. parahemolyticus* can cause considerable losses to shrimp hatcheries worldwide [3].

The whiteleg shrimp’s immune systems are also essential for the emergence of a disease in the aquaculture environment. Crustaceans, especially shrimp, only have a nonspecific and humoral natural immune system [4]. This...
causes the growth and immunity of shrimp to be increased, depending on the quality of the broodstock, the environment, and the application of biocontrol genes [5,6].

Currently, antibiotics and chemotherapy agents are still used to control bacterial diseases [7,8]. However, the use of antibiotics unwisely can lead to the accumulation of residues in the tissue, resulting in a decrease in product quality, the emergence of antibiotic resistance problems in shrimp and other aquatic organisms [9], and pollution of the aquatic environment [10]. Therefore, the use of environmentally friendly materials is needed to control diseases in the current aquaculture system. Several approaches, such as the use of pro-, pre-, and synbiotics [11], immuno-stimulants [12], vaccination [13], quorum-sensing, phage application [14], RNA interference [15], development of molecular-based diagnostic materials, and the breeding and spawning of specific pathogen-free shrimp [16], have attracted the attention of researchers and shrimp farmers. Phytobiotics are functional additives derived from nature, including food supplement ingredients that have become an alternative to improve health and resistance to disease attacks that are ecologically correct in the modern aquaculture sector [17]. The development of phytobiotic materials is the best effort to improve the shrimp immune system. In addition to being compatible with the shrimp immune system, which is still primitive (nonspecific immunity), phytobiotics also have several advantages, such as abundant sources of materials, a wide target range, the potential for large-scale application, and environmental friendliness. Some of the natural agents that can be used as phytobiotics to increase the immune system of whiteleg shrimp are hairy eggplant (Solanum ferox) and bitter ginger (Zingiber zerumbet) [18]. Hairy eggplant, or S. ferox, is widely planted in tropical areas such as Indonesia for its fruit. This plant has been listed as a medicinal plant in the ethnobotanical inventory because it is reported to be effective in treating human diseases. Hairy eggplants contain bio-active compounds such as phenolics, flavonoids, and polyphenols that play an essential role in preventing oxidative stress and several biological effects such as antioxidants and anti-inflammatory [19]. Another traditional plant such as Z. zerumbet (L), part of the Zingeribaceae family, is commonly found in Southeast Asia, such as Indonesia. This plant is traditionally used in various cuisines and drinks because it is reported to have anti-allergic properties in its rhizomes. Previous studies have shown that the combination of Z. zerumbet and Curcuma zedoaria added to the orange-spotted grouper (Epinephelus coioides) was able to act as an immunostimulant by increasing nonspecific immune responses (respiratory burst activity, reactive oxygen species, phagocytic activity, superoxide dismutase, and lysozyme activity) [20]. Hardi et al. [21] explained that the combination of Boesenbergia pandurata, Z. zerumbet, and S. ferox effectively prevents infection with Aeromonas hydrophila and Pseudomonas sp., and modulates the immune system of tilapia, Oreochromis niloticus. However, studies highlighting the potential of phytobiotics have not been reported in white shrimp, although compound derivatives of phytobiotics have been previously reported [22].

Based on this report, this study aimed to evaluate the combination of hairy eggplant (S. ferox) and bitter ginger (Z. zerumbet) in increasing the production performance and immune system of whiteleg shrimp (L. vannamei). This study is expected to provide valuable preliminary information for applying environmentally friendly immunostimulants to increase Pacific white shrimp production.

Materials and Methods

Ethical approval

This research has been licensed by the Fisheries Expert Polytechnic (AUP), Marine and Fisheries Research and Human Resources Agency (BRSDM), Ministry of Marine Affairs and Fisheries (KKP) of the Republic of Indonesia with No: 1832/POLTEK-AUP/TU.210/IX/2020.

Study area and period

This study was conducted from September 2020 to December 2020 at the Loka Pengelolaan Sumber Daya Pesisir and Laut Serang, Directorate General of Marine Space Management, Ministry of Marine and Fisheries, Republic of Indonesia.

Study design

This study used a completely randomized design with four treatments and three replications. The treatments used were a combination of hairy eggplant (S. ferox) and bitter ginger (Z. zerumbet) with the following details:

- P1: no combination + vitamin C (control);
- P2: the combination of S. ferox and Z. zerumbet with a dose of 120 ml/l;
- P3: the combination of S. ferox and Z. zerumbet with a dose of 100 ml/l;
- P4: the combination of S. ferox and Z. zerumbet with a dose of 80 ml/l.

Preparation of S. ferox and Z. zerumbet

For preparation of the extract, we referred to the study by Hardi et al. [21]. In short, the plants were collected from traditional markets. The materials were washed and dried in an oven at 40°C–45°C for 48 h. The dry ingredients were then ground using a blender to form a fine powder. Exactly 100 gm of dry samples were mixed with 100 ml of 96% ethanol in an Erlenmeyer flask at room temperature for
Rearing of whiteleg shrimp

Rearing of whiteleg shrimp was carried out in a round tarpaulin container with a pool diameter of 2 m and a height of 1 m, for a total of 12 pools. Each pond has nine aeration points evenly distributed in the rearing pond (Fig. 1). The extract was mixed into the feed and the dose was adjusted for each treatment. Feeding was carried out four times a day at 07.00 a.m., 11.00 a.m., 3.00 p.m., and 7.00 p.m., respectively. The amount of feed given was adjusted to the feeding rate according to the Standard National Indonesia No. 01-7246-2006, namely 15%–10%.

Production performance parameters

Survival rate (SR)

The SR of whiteleg shrimp was calculated at the end of the rearing period. The calculation was carried out according to the equations described in Wiradana et al. [23] and Fendjalang et al. [24] as follows:

\[
SR\% = \frac{N_f}{N_0} \times 100\%
\]

Where
SR: Survival rate (%);
Nf: Number of shrimp at the end of the study (individual);
N0: Number of shrimp at the beginning of the study (individual).

Absolute weight growth

Weight growth was observed every 7 days by weighing the number of shrimp. Absolute weight growth based on Widanarmi et al. [25] is as follows:

\[
\Delta W = W_f - W_0
\]

Where
\(\Delta W\): Absolute weight growth (gm);
Wf: Final shrimp weight (gm);
W0: Initial shrimp weight (gm).

Specific growth rate (SGR)

The SGR was observed once a week based on Zubaidah et al. [26] as follows:

\[
SGR\% \text{day} = \frac{\ln W_f - \ln W_0}{T} \times 100\%
\]

Note:
SGR(%/day): Specific growth rate;
Wf: Final shrimp biomass (gm);
W0: Initial shrimp biomass (gm);
T: Rearing period (days).

Feed conversion ratio (FCR)

FCR was calculated at the end of the rearing period based on Sarjito et al. [27] as follows:

\[
FCR = \frac{\text{Weight Gain}}{\text{Feed Consumption}}
\]
The whiteleg shrimp immune response was evaluated by calculating the total hemocyte count (THC) and differential hemocyte counts (DHC). Collection and preparation of shrimp hemolymph were carried out by Zahra et al. [28]. THC and DHC were analyzed by taking 0.1 ml of hemolymph in the fifth pereopod using a 1 ml syringe filled with 0.3 ml of Na-EDTA anticoagulant to prevent blood clots. The sample was homogenized for 5 min in a moistened microtube with 10% Na-EDTA. Hemolymph was dripped on the hemocytometer and closed using a cover glass. Calculation of the number and type of cells was carried out under a light microscope with a magnification of 400×. Observation of immune parameters was carried out under a light microscope. The calculation of THC and DHC refers to the method by Suleman et al. [29] as follows:

\[
\text{THC} = \frac{\text{average } \ell \text{ counted cells } \times 250 \times \text{dilution factor} \times 1,000}{L/B}
\]

\[
\text{DHC} (%) = \frac{\ell A}{L B} \times 100
\]

**Water quality**

To maintain the media quality, daily draining of water was carried out [28]. Water quality parameters measured include temperature, dissolved oxygen (DO), pH, ammonia, nitrate, and nitrite. Water quality parameters are used as supporting data in this study.

**Immunity parameters**

The whiteleg shrimp immune response was evaluated by calculating the total hemocyte count (THC) and differential hemocyte counts (DHC). Collection and preparation of shrimp hemolymph were carried out by Zahra et al. [28]. THC and DHC were analyzed by taking 0.1 ml of hemolymph in the fifth pereopod using a 1 ml syringe filled with 0.3 ml of Na-EDTA anticoagulant to prevent blood clots. The sample was homogenized for 5 min in a moistened microtube with 10% Na-EDTA. Hemolymph was dripped on the hemocytometer and closed using a cover glass. Calculation of the number and type of cells was carried out under a light microscope with a magnification of 400×. Observation of immune parameters was carried out under a light microscope. The calculation of THC and DHC refers to the method by Suleman et al. [29] as follows:

\[
\text{THC} = \frac{\text{average } \ell \text{ counted cells } \times 250 \times \text{dilution factor} \times 1,000}{L/B}
\]

\[
\text{DHC} (%) = \frac{\ell A}{L B} \times 100
\]

**Histological analysis**

The gill tissue was placed in Davidson’s solution until dehydrated, embedded in paraffin, and sliced using a microtome. The tissue was stained using hematoxylin and eosin and analyzed to identify defects such as hyperplasia, vacuolation, and necrosis. The histology is shown in the figures defined by Mari et al. [30].

**Data analysis**

Production performance parameters and immunity were tabulated using MS Excel 2019 (Microsoft, USA). Statistical analysis was carried out using Statistical Package for the Social Sciences Version 25 software (IBM, USA). The data underwent a homogeneity test, followed by a single-factor analysis of variance with a 95% confidence interval. To find out the differences in each treatment, the data underwent Duncan’s test. The statistical results were interpreted and presented in the form of tables and figures.

**Results and Discussion**

**Production performance**

The supplementation of hairy eggplant and bitter ginger in feed can increase absolute weight growth in whiteleg shrimp. The highest absolute weight growth was 9.19 gm/individual and was found in P3, then in P4 (6.97 gm/individual), P2 (6.03 gm/individual), and P1 (control) (5.82 gm/individual) (Table 1). Weight gain can occur from energy entering the body. The energy obtained from feed ingredients will first be used for rearing activities, then the remaining energy will be used for the growth process [31,32]. The absolute weight growth of shrimp with hairy eggplant and bitter ginger was higher than the control whiteleg shrimp. This occurs due to the supplementation of hairy eggplant and bitter ginger, which can stimulate the absorption of feed nutrients, mainly due to their protein

### Table 1. The results of the analysis of the production performance parameters of whiteleg shrimp (L. vannamei).

| Treatments group | Absolute growth (g) | SGR (%) | FCR | SR (%) |
|------------------|---------------------|---------|-----|--------|
| P1               | 5.82 ± 0.12<sup>a</sup> | 5.49 ± 0.04<sup>a</sup> | 1.51 ± 0.05<sup>a</sup> | 71.92 ± 1.76<sup>a</sup> |
| P2               | 6.03 ± 0.06<sup>a</sup> | 5.67 ± 0.07<sup>a</sup> | 1.45 ± 0.03<sup>a</sup> | 76.95 ± 3.03<sup>a</sup> |
| P3               | 9.19 ± 0.52<sup>a</sup> | 6.69 ± 0.18<sup>c</sup> | 0.90 ± 0.08<sup>b</sup> | 90.66 ± 4.61<sup>b</sup> |
| P4               | 6.97 ± 0.06<sup>b</sup> | 5.93 ± 0.03<sup>b</sup> | 1.27 ± 0.01<sup>b</sup> | 77.09 ± 1.40<sup>b</sup> |

SGR: Specific growth rate; FCR: Feed conversion ratio; SR: Survival rate.

The mean ± standard deviation values with different superscript letters show significantly different results (<p> < 0.05).
and bioactive content. Bioactive content such as flavonoids in traditional plants can act as antibacterials and antioxidants, minimizing the increase in pathogenic microflora in the digestive tract. It is thought to increase the digestibility of whiteleg shrimp [33–35].

The best SGR was found in P3 with a growth rate of 6.69%/day, showing that the doses of hairy eggplant and bitter ginger had significant differences ($p < 0.05$) in SGR on whiteleg shrimp. Increased growth rates can occur due to optimal use of feed ingredients. On the other hand, a low growth rate can occur due to health problems, stress, and suboptimal utilization of feed nutrients used for growth [36].

The lowest FCR value was found in P3, then in P2 and P4, respectively. The highest FCR (1.51) was found in the control treatment (Table 1). The FCR was inversely proportional to weight growth, so the lower the FCR, the higher the efficiency of the shrimp in utilizing feed for growth [37]. On the other hand, if the shrimp body is unstable, then the shrimp may experience a decrease in appetite and the feed provided is not converted into biomass. Stressed or unhealthy shrimp can be better at converting feed ingredients for weight growth compared to healthy shrimp [38].

The highest SR was found in P3 (90.66%), and out of all treatments, the lowest percentage was found in the control treatment (71.92%) (Table 1). This indicated that supplementation of hairy eggplant and bitter ginger on whiteleg shrimp had a significant difference in the SR of whiteleg shrimp maintained for 56 days. This is presumably due to the content of secondary metabolites in hairy eggplant and bitter ginger, which can increase the immune system against pathogenic bacterial infections to protect the shrimp body from stress. The biological process will increase due to the involvement of phytochemical substances produced by the extract ingredients, which are capable of producing enzymes for detoxification, modulating the immune system, and increasing shrimp survival. The increased immune response can have a positive impact on increasing body resistance and reducing shrimp mortality. This is confirmed by Jasmanindar et al. [39], who found that the low SR in the treatment without the extract had a relationship with a weak immune system compared to the treatment with seaweed extract. The combination of phytobiotics (thyme essential oil, red thyme, and rosemary pepper) applied in a supplement for 20 days has significant benefits for improving antioxidant protection, reducing the impact of stressors, and modulating the immunity of tilapia against *A. hydrophila* infection [17].

### Water quality

All variables observed in the rearing pond remained within the levels recommended for shrimp culture during the test period (Table 2).

### Immunity parameters

The hemato-immunological response is a central physiological mechanism, playing a role in protecting animals from disease, environmental stressors, or specific biological agents [40,41], such as phytobiotics. In this study, THC and DHC were carried out to evaluate the immunity condition of whiteleg shrimp with hairy eggplant and bitter ginger during 56 days of rearing. Based on the results, the total hemocytes of whiteleg shrimp in all treatments during the rearing period was around $4.63–16.76 \times 10^6$ cells/mm$^3$. The highest THC value was found in P3 and was significantly different from the other three treatments ($p < 0.05$). P1 had the lowest THC value of all treatments. P2 and P3 had no significant differences (Table 3).

Hemocytes are the primary mediators of cellular responses in crustaceans, with roles that include self-recognition, phagocytosis, production of reactive oxygen intermediates, wound healing, and the process of melanization by encapsulation of foreign materials [42–44]. The increase in THC of whiteleg shrimp with hairy eggplant and bitter ginger can be suspected due to the effect of bioactive compounds that can modulate shrimp immunity. Other studies confirmed that natural hydrolyzed tannin products from sweet chestnut (*Castanea sativa*) could act as functional feed additives by promoting growth and hematological parameters of whiteleg shrimp [45]. The *in-vivo* effects of Astragalus polysaccharide immunostimulating ingredients, chlorogenic acid, and berberine showed a higher increase in THC in whiteleg shrimp [46].

The difference in THC values in each treatment may be due to the various concentrations of active ingredients in food due to the treatment that has been determined. The low value of THC at P1 (control) may be influenced by physiological factors such as the slow formation of hemocytes in the shrimp body [47,48]. The exciting discussion confirmed that the low number of shrimp hemocytes is due to infiltration of regenerated tissue and hemocyte cell death due to apoptosis [27].

### Table 2. Water quality parameters during the maintenance period.

| Parameter | This study | Optimal range$^a$ |
|-----------|-----------|------------------|
| pH        | 6.5–8.5   | 7.5–8.5          |
| Temperature (°C) | 27–33 | 28–30 |
| DO (mg/l) | 3.8–4.0   | ≥ 4              |
| Ammonia (mg/l) | 0–0.1 | ≤ 0.1 |
| Nitrite (mg/l) | 0–0.52 | ≤ 1          |
| Salinity  | 29.7–33   | 26–32            |
| Nitrate (mg/l) | 0–0.3 | 0.5          |

$^a$ Ministry of Marine Affairs and Fisheries Regulation (KKP), Republic of Indonesia, 2016.
The minor component of shrimp hemocytes in the DHC is hyaline, while the other two types of hemocytes are granular and semi-granular cells. The highest percentage of hyaline was found in P3 (48.67%), followed by P4 (34.67%), P2 (31.33%), and P1 (24.33%) (Table 3). The lowest number of semi-granular cells was found in P3 (23.67%). The decreasing number of semi-granular cells was shown in all treatments, except control (P1), with the highest number (Table 3). The same thing happened to the number of granular cells in the treatment group. The highest number of granular cells was found in P1/control (40.33%).

Hyaline cells have a vital role in the shrimp’s defense system. This cell type has a high ratio of cytoplasmic nuclei and few cytoplasmic granules. An increase in the number of hyaline cells can be associated with phagocytic activity when in contact with antigens or immunostimulating substances that will stimulate the body’s defense activity to evoke the first defense response [49]. Semi-granular cells have a relationship with the addition or reduction of hyaline cells; so, the decrease in the number of semi-granular cells in the treatment group was due to the process of further development into hyaline cells. As a result, these cells cannot develop into semi-granular cells, so the number of semi-granular cells is low. Semi-granular cells are more involved in the encapsulation mechanism. The encapsulation process is a defense reaction against a large number of foreign particles that cannot be phagocytized by hyaline cells. These cells respond more to polysaccharide compounds found in bacterial cell walls.

Granular cells are the type of hemocytic cells that have the most significant size with an active nucleus in the storage process until the release of prophenoloxidase and cytotoxicity. In this study, an increase in granular cells also occurred in the control group (P1). This is due to the low number of hyaline cells involved in the first defense process, thus relying on granular cells for nonspecific body defense, which is driven by the influence of immunostimulatory components such as vitamin C.

**Histology analysis**

The condition of gill tissue on whiteleg shrimp showed that treatments P1 (control) and P3 experienced vacuolation and hyperplasia (Fig. 2), followed by treatments P2, which only experienced hyperplasia, and treatment P4, which experienced vacuolation and necrosis.

The histology can explain that gill tissue damage occurred in all treatments carried out. Physiological differences between each whiteleg shrimp can cause tissue damage even when extracted ingredients are used. More research is needed to prove these results. High hemocytes can indicate infection or stress factors. Clogged blood flow in the lamellae (due to physical trauma, pollutants, or other physiological disturbances) can cause edema (cell swelling) between the blood vessels and the epithelial lining of the primary lamellae. Miller and Zachary [50] explained that necrosis is acute cell damage and can be massive, resulting in incomplete tissue formation due to shrinkage or complete shrinkage of the nucleus. Hyperplasia is the formation of excessive tissue due to an increase in the number of cells so

| Treatment Groups | THC ($\times$ 10$^3$ cells/mm$^3$) | Hyaline (%) | Semi Granular (%) | Granular (%) |
|------------------|-------------------------------|-------------|-------------------|--------------|
| P1               | 4.63 ± 2.05$^b$               | 24.33 ± 2.08$^a$ | 35.33 ± 1.52$^c$ | 40.33 ± 0.57$^d$ |
| P2               | 8.63 ± 0.87$^c$               | 31.33 ± 1.52$^b$ | 31.00 ± 1.00$^b$ | 37.67 ± 0.57$^c$ |
| P3               | 16.76 ± 0.90$^c$             | 48.67 ± 4.04$^c$ | 23.67 ± 3.51$^a$ | 27.67 ± 0.57$^a$ |
| P4               | 9.96 ± 1.75$^c$              | 34.67 ± 1.52$^c$ | 30.33 ± 0.57$^a$ | 35.00 ± 1.73$^a$ |

THC: Total hemocyte counts.
The mean values with different superscript letters show significantly different results ($p < 0.05$).

![Figure 2](image-url)
that lamellae with hyperplasia will experience thickening of epithelial tissue at the ends of the filaments or the epithelium located near the base of the gill lamellae [51].

Based on the results, a 100 ml/l combination of hairy eggplant and bitter ginger in the feed is auspicious for whiteleg shrimp, related to their production performance and immunity. However, further studies are still needed to emphasize the potential that this combination of extracts can provide against pathogenic bacteria and viruses in whiteleg shrimp.

Conclusion

The supplementation of hairy eggplant and bitter ginger into the feed affected the production performance of whiteleg shrimp as indicated by absolute weight growth, SGR, low FCR, and high survival as shown in P3 (dose of 100 ml/l). Similarly, the immunity parameters, including THC and DHC, were high in the P3 treatment group, although there was a tendency for higher semi-granular and granular cell values in the control group. All these results indicate that the combination of hairy eggplant and bitter ginger as phytobiotic ingredients could act as an exemplary modulator of the nonspecific immune response of whiteleg shrimp, which ultimately increases production performance. Further studies are needed to obtain valuable information regarding the combination of this extract against infection with whiteleg shrimp pathogens, such as viruses and bacteria, to be used as a preventive agent.

Acknowledgment

This research was funded by a grant from the Polytechnic College of Fisheries Experts (AIP), Jakarta, Indonesia. The authors would like to thank the head and staff of the Loka Pengelolaan Sumber Daya Pesisir and Laut (PSPL) Serang, Directorate General of Marine Space Management, Ministry of Marine and Fisheries, Republic of Indonesia, who have given us permission to carry out this research.

List of Abbreviations

a.m., ante meridiem; °C, degrees Celsius; DO, dissolved oxygen; DHC, differential hemocytes counts; FCR, feed conversion; gm, gram; l, liter; ml, milliliter; µm, micrometer; p.m., post meridiem; SGR, specific growth rate ratio; SR, survival rate; THC, total hemocyte count.

Conflict of interest

The authors declare no conflict of interest.

Authors’ contribution

SR designed the study, supervised, and conducted critical checking of this manuscript. DR interpreted the results and reviewed the manuscript. MATV conducted the study, collected the data, and contributed to manuscript preparation. PAW drafted the manuscript, took part in preparing it, and performed critical checking of the manuscript. All authors read and approved the final manuscript.

References

[1] FAO. The state of food in security in the world 2015: meeting the 2015 international hunger targets: taking stock of un even progress. FAO, 2016. Available via http://www.fao.org/stateoffoodinsecurityinteworld2015/report/ (Accessed 8 February 2021).
[2] Sani MD, Maharani AY, Riyandi MI, Joko R, Susilo K, Wiradana PA, et al. Monitoring of population density of Vibrio sp. and health condition of hepatopancreas Pacific white shrimp (Litopenaeus vannamei) cultivated with intensive systems in Bulukumba Regency, South Sulawesi, Indonesia. Ecol Environ Conserv 2020; 26(3):1271–5.
[3] Zhu XF, Guo H, Li GL, Zhu CH. Effects of dietary hydrolyzable tannins on growth performance, antioxidant capacity, intestinal microflora and resistance against Vibrio parahaemolyticus of juvenile Pacific white shrimp. Litopenaeus vannamei (Boone, 1931). Aquac Reports 2021; 19:100601; https://doi.org/10.1016/j.aqrep.2021.100601
[4] Dewi NN, Krismiyati K, Rozi R, Maharsi G, Satyantini WH. Application of probiotics, immunostimulants, and water quality management in an effort to increase production of vannami shrimp (Litopenaeus vannamei) in Ujung Panjang District, Gresik Regency, Kabupaten Gresik (In Bahasa). J Aquac Fish Heal 2019; 8(3):178–83; https://doi.org/10.20473/jafh.v8i3.15127
[5] Hidayat RP. Evaluation of provision of Zanthonniun Pennaei crude protein on growth rate, immune response and survival of vannami shrimp (Litopenaeus vannamei) in ponds. J Biosains Pascasarj 2017; 19(2):112–32; https://doi.org/10.20473/jbp.v19i2.2017.111-126
[6] Zhu XF, Guo H, Li GL, Zhu CH. Effects of dietary hydrolyzable tannins on growth performance, antioxidant capacity, intestinal microflora and resistance against Vibrio parahaemolyticus of juvenile Pacific white shrimp. Litopenaeus vannamei (Boone, 1931). Aquac Reports 2021; 19:100601; https://doi.org/10.1016/j.aqrep.2021.100601
[7] Zhang Z, Yang Z, Wang Y, Liao M, Wei X, Yu YX, et al. Effectiveness of garden burnet, Sanguisorba officinalic L., in controlling acute hepatoencephalic necrosis disease caused by infection of Vibrio parahaemolyticus in shrimp farming. Aquaculture 2021; 531:735–875; https://doi.org/10.1016/j.aquaculture.2020.735875
[8] Thornber K, Verner-Jeffreys D, Hinchliffe S, Rahman MM, Bass D, Tyler CR. Evaluating antimicrobial resistance in the global shrimp industry. Rev Aquac 2020; 12(2):966–86; https://doi.org/10.1111/rqa.12367
[9] Kathleen MM, Samuel L, Felecia C, Reagan EL, Kasing A, Lesley M, et al. Antibiotic resistance of diverse bacteria from aquaculture in Borneo. Int J Microbiol 2016; 2016:1–9; https://doi.org/10.1155/2016/2164761
[10] Arisa II, Widanarni W, Yuhana M, Muchlisin ZA, Abdullah AA. The application of probiotics, prebiotics and symbiotics to enhance the immune responses of vannami shrimp (Litopenaeus vannamei) to Vibrio harveyi infection. AACL Bioflux 2015; 8(5):772–8.
[11] Wang W, Sun J, Liu C, Xue Z. Application of immunostimulants in aquaculture: current knowledge and future perspectives. Aquac Res; 2017; 48(1):1–23; https://doi.org/10.1111/are.13161

[12] Amatul-Samahah MA, Wan Omar WHH, Mohd Ishak NF, Amal Azmni MA, Zamri-Saad M, Ina-Salwany MY. Vaccination trials against vibrois in shrimp: a review. Aquac Res 2020; 18:100471; https://doi.org/10.1007/s11160-020-10047-1

[13] Chen L, Fan J, Yan T, Liu Q, Yuan S, Zhang H, et al. Isolation and characterization of specific phages to prepare a cocktail preventing Vibrio sp. Va-F3 infections in shrimp (Litopenaeus vannamei). Front Microbiol 2019; 10(1):23–37; https://doi.org/10.3389/fmicb.2019.00235

[14] Nguyen DV, Christiaens O, Bossier P, Smaghe G. RNA interference in shrimp and potential applications in aquaculture. Rev Aquac 2018; 10(3):573–84; https://doi.org/10.1016/j.raaq.12187

[15] Alday-Sanz V, Brock J, Flegel TW, McIntosh R, Bondad-Reantaso MG, Salazar M, et al. Facts, truths and myths about SPE shrimp in aquaculture. Rev Aquac 2018; 12(1):76–84; https://doi.org/10.1011/raaq.12305

[16] Setyawan A. Fucoidan from tropical brown algae as immunostimulant of vannamei shrimp (Litopenaeus vannamei): study and application. PLoS One 2015; 10(8):e0135783; https://doi.org/10.1371/journal.pone.0135783

[17] Oszmiański J, Kolniak-Ostek J, Wojdyło A. Characterization of phe nolic compounds and antioxidant activity of Solanum scabrum and Solanum burbancki berries. J Agric Food Chem 2014; 62(7):1512–1519; https://doi.org/10.1021/jf4045233

[18] Rahman RA, Kendall G, Ramli R, Jamari Z, Ku-Mahamud KR. Shrimp feed formulation via evolutionary algorithm with power heuristics for handling constraints. Complexity 2017; 2017(11):1–12; https://doi.org/10.1155/2017/7053710

[19] Uyun S, Damayanti AA, Azhar F. The effect of cherry leaves extract (Muntingia calabura) on growth performance of white shrimp (Litopenaeus vannamei). J Biol Trop 2021; 21(1):262–70; https://doi.org/10.29033/jbtv.2211.2450

[20] Soonthornchai W, Chaiyapechara S, Jarayabhand P, Söderhäll K. Acute phase marker and its utilization on survival and growth of Pacific white shrimp (Litopenaeus vannamei). J Biol Trop 2021; 21(1):1–3; https://doi.org/10.1088/1748-9326/11(6):1–31

[21] Rahman RA, Kendall G, Ramli R, Jamari Z, Ku-Mahamud KR. Shrimp feed formulation via evolutionary algorithm with power heuristics for handling constraints. Complexity 2017; 2017(11):1–12; https://doi.org/10.1155/2017/7053710

[22] Chandran MN, Moovendhan S, Suganya AM, Tamikselvi A, Bebin, Immanuel G, et al. Influence of polyherbal formulation (Aqualimu) as a potential growth promoter and immunomodulator in shrimp Peneaus monodon. Aquac Reports 2016; 4:143–9; https://doi.org/10.1016/j.aqrep.2016.10.002

[23] Ayisi CI, Hua X, Apraku A, Afriyie G, Ijey BA. Recent studies toward the development of practical diets for shrimp and their nutritional requirements. HAYATI J Biosci 2017; 24(3):109–17; https://doi.org/10.1016/j.jhjb.2017.09.004

[24] Fry JP, Mailloux NA, Love DC, Milli MC, Cao L. Feed conversion efficiency in aquaculture: do we measure it correctly? Environ Res Lett 2018; 13(2):024017; https://doi.org/10.1088/1748-9326/aaa273

[25] Aqillah-Amr MA, Hidir A, Azra MN, Ahmad-Ideris AR, Abualreesh AMH, Noordiyana MN, et al. Use of pelleted diets in commercially farmed decapods during juvenile stages: a review. Animals 2021; 11(6):1–31; https://doi.org/10.3390/ani11061761

[26] Huang Z, Aweya JJ, Zhu C, Tran NT, Hong Y, Li S, et al. Modulation of crustacean innate immune response by amino acids and their metabolites: inferences from other species. Front Immunol 2020; 11(6):1–31; https://doi.org/10.3389/fimmu.2020.057472

[27] Jiravanichpaisal P. Interaction of algal extracts on total haemocyte count and phagocytic activity of L. vannamei. J Vet Life Sci 2018; 5(10):1–12; https://doi.org/10.21776/ub.rjls.2018.005.03.3

[28] Zahra A, Sukenda S, Wahjuningrum D. Extract of seaweed Gracilaria verrucosa as immunostimulant to controlling white spot disease in Pacific white shrimp Litopenaeus vannamei. J Akuakultur Indonesia 2017; 16(2):174–83; https://doi.org/10.19027/jai.2016.174-183

[29] Rahman RA, Kendall G, Ramli R, Jamari Z, Ku-Mahamud KR. Shrimp feed formulation via evolutionary algorithm with power heuristics for handling constraints. Complexity 2017; 2017(11):1–12; https://doi.org/10.1155/2017/7053710

[30] Zafar K, Rehman I, Jabeen S, Rehman MA, Zafar I, et al. Further studies on the development of practical diets for shrimp and their nutritional requirements. HAYATI J Biosci 2017; 24(3):109–17; https://doi.org/10.1016/j.jhjb.2017.09.004

[31] Alimuddin A, Junior MZ, Utomo NBP, Alimuddin A, Junior MZ, Utomo NBP. The chemical composition of Gracilaria verrucosa extract and its utilization on survival and growth Litopenaeus vannamei. Oman-Akuatika 2018; 14(3):1–9; https://doi.org/10.20884/1.ooa.2018.143.508

[32] Chen Y, Sim SS, Chiew SL, Yeh ST, Liou CH, Chen JC. Dietary administration of a Gracilaria tenusitipta extract produces protective immunity of white shrimp Litopenaeus vannamei in response to ammonia stress. Aquaculture 2012; 370–371(12):26–31; https://doi.org/10.1016/j.aquaculture.2012.09.031

[33] Chen Y, Sim SS, Chiew SL, Yeh ST, Liou CH, Chen JC. Dietary administration of a Gracilaria tenusitipta extract produces protective immunity of white shrimp Litopenaeus vannamei in response to ammonia stress. Aquaculture 2012; 370–371(12):26–31; https://doi.org/10.1016/j.aquaculture.2012.09.031

[34] Martin GG, Poole D, Poole C, Hose JE, Arias M, Reynolds L, et al. The role of the immune system in the development of practical diets for shrimp and their nutritional requirements. J Appl Vet Anim Res; 2018; 9(1):78–86, March 2022

[35] Soonthornchai W, Chaiyapechara S, Jarayabhand P, Söderhäll K. Acute phase marker and its utilization on survival and growth Litopenaeus vannamei. J Biol Trop 2021; 21(1):1–3; https://doi.org/10.1088/1748-9326/11(6):1–31

[36] Rahman RA, Kendall G, Ramli R, Jamari Z, Ku-Mahamud KR. Shrimp feed formulation via evolutionary algorithm with power heuristics for handling constraints. Complexity 2017; 2017(11):1–12; https://doi.org/10.1155/2017/7053710

[37] Alimuddin A, Junior MZ, Utomo NBP, Alimuddin A, Junior MZ, Utomo NBP. The chemical composition of Gracilaria verrucosa extract and its utilization on survival and growth Litopenaeus vannamei. Oman-Akuatika 2018; 14(3):1–9; https://doi.org/10.20884/1.ooa.2018.143.508

[38] Chen Y, Sim SS, Chiew SL, Yeh ST, Liou CH, Chen JC. Dietary administration of a Gracilaria tenusitipta extract produces protective immunity of white shrimp Litopenaeus vannamei in response to ammonia stress. Aquaculture 2012; 370–371(12):26–31; https://doi.org/10.1016/j.aquaculture.2012.09.031

[39] Martin GG, Poole D, Poole C, Hose JE, Arias M, Reynolds L, et al. Clearance of bacteria injected into the hemolymph of the penaeid shrimp, Scyonia ingentis. J Invertebr Pathol 1993; 62(3):308–15; https://doi.org/10.1007/jpa.1993.1118
Ahmed et al. / J. Adv. Vet. Anim. Res., 9(1): 78–86, March 2021