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Posted Date: 2 August 2024

doi: 10.20944/preprints202408.0052.v1

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Article

Effects of Fermented Herbal Extract as Phytobiotic on Growth Indices, Moulting Performance, and Feed Utilization of Juvenile Tiger Shrimp (*Penaeus monodon* Fabr.)

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Abstract: The objective of this study was to investigate the impact of adding fermented herbal extracts (FHE) derived from mulberry leaf (*Morus alba*), Javanese turmeric (*Curcuma xanthorrhiza*), and fingerroot (*Boesenbergia rotunda*) to the diet of tiger shrimp (*Penaeus monodon*) on their growth, moulting performance, feed efficiency, and nutrient retention. The main feed used in this trial was a commercially manufactured pellet, then five different doses of FHE supplementation were used: 0 mg/kg feed (P0, control), 50 mg/kg feed (P1), 100 mg/kg feed (P2), 150 mg/kg feed (P3), and 200 mg/kg feed (P4). Weight gain, average daily gain, and length gain of shrimps fed P2 was significantly higher than that of those fed the control diet. Similar result was observed in moulting performance. The application of P2 showed superior results in enhancing the FE of cultured shrimp. Thus, the protein and energy retention of *P. monodon* was significantly better in P2 treatment groups. P0 had the lowest crude protein, while shrimp given P2 and P3 diets had the highest crude protein content of any treatment group. Based on the findings, it is recommended that the juvenile tiger shrimp diet contain 100 mg/kg of FHE for the best effects.

Keywords: fermented herbal extract; growth; moulting; nutrient retention; tiger shrimp

Key Contribution: The current investigation recorded that the addition of FHE via the top-coating method enhanced the growth and feed utilization of juvenile *P. monodon*. Utilizing FHE as a phytobiotic at 100 mg/kg diet has advantages in enhancing aquaculture productivity of tiger shrimp; which encompasses the potential to promote the sustainable expansion of the aquaculture sector in the future.

1. Introduction

According to FAO [1], the global output of aquatic animals in 2022 reached a record-breaking 185 million tons (live weight equivalent), representing a 4% increase compared to 2020. Aquaculture yielded around 94 million tonnes of aquatic animals, accounting for 51% of the overall production. This surpassed the production of catch fisheries, which amounted to 91 million tonnes (49%) for the first time. Aquaculture is also vital to food security in underdeveloped nations by creating jobs and increasing nutrition [2,3]. Shrimp farming has emerged as a significant component of the global aquaculture sector, at least in the past two decades, whereas wild shrimp catches have remained

stagnant. Aquaculture's contribution to the global shrimp supply exceeds 83.7% due to the scarcity or decline of wild populations [4]. The surge in output mostly stems from the escalating demand fueled by population and economic expansion, as well as a growing inclination towards nutritious food. *Litopenaeus vannamei* has been the predominant species in this process. The global production of *L. vannamei* has shown significant growth, rising from 155 thousand tonnes in 2000 to 5.8 million tonnes in 2020 [5]. Conversely, the formerly dominant species, *Penaeus monodon*, has not undergone similar expansion and has maintained a relatively stable level of production. Its production has only increased from 631 thousand tonnes in 2000 to 774 thousand tonnes in 2019 [3,6].

Today, the shrimp production sector has implemented innovative approaches, advanced technology, and refined strategies in farm and feed management to guarantee a more sustainable future for the industry compared to its previous practices [7]. Improvements in feed management include the usage of feed additives, which have been demonstrated to have a range of beneficial effects. Feed additives are substances, both nutritive and non-nutritive, that are added to the diet in small amounts. They serve various purposes, including improving the nutritional value of aquafeed, boosting the immune system and reducing stress, making the feed more appealing, promoting growth, and preserving water quality.

Herbal extracts, which are derived from plants, are a potential choice among the many types of feed additives in the aquafeed business [8,9]. They have been extensively employed as prophylactic alternatives to conventional methods of production enhancement. Plant extracts have been reported to favor various functions, such as enhancing the nutritional value of aquafeed, enhancing feed palatability, improving growth performance [10], increasing immunomodulation and stress-alleviation, and maintaining water quality in fish and shrimp aquaculture. However, the use of plant-derived substances in aquafeed to achieve sustainable shrimp farming is influenced by a number of issues, including the presence of antinutritional factors, decreased nutrient bioavailability, indigestible particles, and microbial contamination [11]. Fermentation has garnered significant attention in recent years as a solution to these issues in aquafeed constituents, as it offers host organisms health-promoting probiotic benefits. Multiple studies have demonstrated that fermentation enhances the accessibility and absorbability of nutrients in feed, leading to improved palatability and digestibility [12–14]. Additionally, fermentation eliminates anti-nutritional compounds present in dietary feed ingredients, making them more readily digestible. Ultimately, these improvements in digestion contribute to enhanced growth and overall health performance of aquaculture species.

In light of the growing interest in using herbal feed additives to enhance fish growth and survival in culture systems, the present study investigated the effects of fermented herbal extract (FHE) supplementation in the diets for juvenile tiger shrimp *P. monodon*. This study showcased the feasibility of utilizing eco-friendly alternatives to preserve growth and enhance feeding performances. It emphasized the advantages of incorporating natural nutritional supplements in aquaculture, shedding light on the potential benefits they offer. Moreover, it offered valuable insights for the development of more efficient and sustainable aquaculture operations.

2. Materials and Methods

2.1. Preparation of Fermented Herbal Extract (FHE) as Phytobiotic

The composition of FHE consisted of three herbal extracts, namely mulberry leaf (*Morus alba*), Javanese turmeric (*Curcuma xanthorrhiza*), and fingerroot (*Boesenbergia rotunda*), all present in similar amounts. These extracts were then fermented using probiotics, specifically *Lactobacillus casei* and *Saccharomyces cerevisiae*. The probiotic strain of *L. casei* was acquired from Yakult® (Tokyo, Japan, intended for human consumption). Meanwhile, commercial baker's yeast, *S. cerevisiae*, in the form of instant dry yeast, was utilized. The FHE used as phytobiotic in this study was prepared by the Marine and Fisheries Biotechnology Laboratory of Hasanuddin University, Makassar, Indonesia [15]. Concisely, 1 mL of diluted *S. cerevisiae*, 1 mL of *L. casei* (4×10^6 cfu/mL), and 1 mL of pre-treated (boiled) molasses were combined with 1 L of distilled water containing 500 mg of herbal extracts

(stock FHE). The fermentation process lasted for 30 days under the conditions of 29°C–32°C. The stock FHE was diluted differently for each treatment: treatment P0 involved adding 100 mL of distilled water, treatment P1 involved adding 10 mL of FHE stock to 90 mL of distilled water, treatment P2 involved adding 20 mL of FHE stock to 80 mL of distilled water, and treatment P3 involved using 30 mL to 70 mL of distilled water, and treatment P4 involved adding 40 mL of FHE stock to 60 mL of distilled water. The chemical and proximal composition of FHE is presented in Table 1.

Table 1. Phytochemical and proximal compositions of fermented herbal extract (FHE)¹.

Phytochemicals ²	Concentration ($\mu\text{g ml}^{-1}$)
Sitosterol	5.50
Alkaloid	56.17
Saponin	0.69
Flavonoid	543.65
Curcumin	0.10
Vitamin and Minerals³	
Vitamin A	0.18
Vitamin C	406.85
Phosphorus (P)	< 0.05
Iron (Fe)	11.62
Calium (K)	1536.81
Calcium (Ca)	172.17
Magnesium (Mg)	219.66
Natrium (Na)	729.31
Zinc (Zn)	0.55
Enzymes⁴	
Amylase	0.122
Lipase	0.875
Protease	0.272

¹100 mL of FHE is equivalent to 500 mg of herbal extract (HE). ² Integrated research and testing laboratory of Gadjah Mada University, Indonesia. ³ Makassar Health Laboratory Centre, Indonesia. ⁴ Biochemical Laboratory of FMIPA, Hasanuddin University, Indonesia.

2.2. Experimental Diet

The primary feed utilized in this study was a commercially produced pellet (Samsung, PT CJ Feed, Jombang, Indonesia). The base feed was coated with FHE using the spraying method, as previously applied [16]. Five supplementation levels of FHE were used in the present study: 0 mg/kg feed (P0, control), 50 mg/kg feed (P1), 100 mg/kg feed (P2), 150 mg/kg feed (P3), and 200 mg/kg feed (P4). Then, the FHE-coated feed was left to dry in the air and then packaged based on the daily feeding quantity. The proximal composition of the base feed used in this experiment is shown in Table 2.

Table 2. Proximal compositions of the base feed.

Proximal Composition	Percentage (%)
Moisture	11.33
Crude Protein (dry weight)	35.48
Crude Lipid (dry weight)	6.81
Ash (dry weight)	13.05
Crude Fibre (dry weight)	3.56

2.3. Shrimp and Experimental Conditions

Healthy tiger shrimp juveniles with an average weight of ± 1.00 g were obtained from the Technical Implementing Unit of Brackishwater Aquaculture (TIUBA) in Takalar, South Sulawesi. The shrimps were acclimatized to ambient laboratory conditions for one week. Subsequently, uniform size of the shrimp juveniles were placed and raised in 15 rectangular outdoor plastic containers ($50 \times 40 \times 25$ cm 3). The density of shrimp in each tank was maintained at 30 juveniles per meter square or 10 individuals per tank, representing five treatment groups in triplicates. A high-pressure air pump provided constant aeration to each tank. Throughout this time frame, the feeding rate equivalent to 8% of their biomass was applied. The feeding occurred at four designated intervals throughout the day: 0700, 1200, 1700, and 2200. Unused feed was collected one hour after feeding and measured to determine the amount of feed consumed.

2.4. Proximal Analysis

The proximate composition of the feeds and whole-body of shrimp (6 shrimps from each replicate) that were fed with the experimental diets was evaluated using the AOAC method [17]. In brief, the samples were subjected to oven-drying at a temperature of 105 °C until a consistent weight was achieved to measure the moisture content and dry matter. The nitrogen content was measured using an automated digestor (Vadopest, Gerhardt, Germany) and a Kjeltec auto distillation unit (Tecator Kjeltec 2200, FOSS, Denmark). The crude protein was then estimated by multiplying the nitrogen concentration by 6.25 after titration. The analysis of crude lipid content was conducted by extracting dried samples using the Soxhlet method with 1.25% H₂SO₄ and 1.25% NaOH as solvents. The extraction was performed using the Soxhlet extractor (Tecator Soxtec 2050, FOSS, Sweden). The fat-free samples underwent acid and base digestion, followed by oven drying, to determine the crude fibre content. To measure the ash content, the samples were incinerated in an electric muffle furnace (Electric PM L6/12, Luoyang, China) at a temperature of 550 °C for a duration of 6 hours. The gross energy of the tested diets was computed using the NRC method [18].

2.5. Growth and Molting Performances

Parameters that were assessed as indices for growth included the weight gain (WG), length gain (LG), average daily gain (ADG) as well as molting performance (MP). Throughout the experiment, WG, FL, ADG and MP were measured according to the following formulae:

$$WG = W_t - W_0 \quad (1)$$

where W_t and W₀ are the final and initial weights, respectively.

$$LG = L_t - L_0 \quad (2)$$

where L_t and L₀ are the final and initial lengths, respectively.

$$ADG = (L_n W_t - L_n W_0) t^{-1} \times 100\% \quad (3)$$

where W_t and W₀ are the final and initial weights, respectively; t is the number of days in the feeding trial.

$$MP = (SS - NS) \times 100\% \quad (4)$$

where SS and TS are the loose shrimp shells and number of shrimp in the tank, respectively.

2.6. Feeding Performances and Nutrient Retention

Feed efficiency (FE) was calculated for all treatment groups using the following formulae:

$$FE = (W_t - W_0) F^{-1} \times 100\% \quad (5)$$

where W_t and W₀ are the final and initial weights, respectively; F is the amount of feed consumed throughout the experiment.

The nutrient retention assessment for protein, lipid, and energy was calculated, as per methods reported previously [15] as follows:

$$PR = ((Wt \times Pt) - (Wo \times Po)) PI^{-1} \times 100\% \quad (6)$$

where Pt and Po are the final and initial protein contents, respectively; PI is the average protein intake during the trial.

$$LR = ((Wt \times Lt) - (Wo \times Lo)) LI^{-1} \times 100\% \quad (7)$$

where Lt and Lo are the final and initial lipid contents, respectively; LI is the average lipid intake during the trial.

$$ER = ((Wt \times Et) - (Wo \times Eo)) EI^{-1} \times 100\% \quad (8)$$

where Et and Eo are the final and initial energy contents, respectively; EI is the average energy intake during the trial.

2.7. Statistical Analysis

The data are reported as the mean \pm standard error (SE) of 3 replicates. The percentage data underwent an arcsin square root transformation. The data were assessed for normal distribution and for equal variance using one-way analysis of variance (ANOVA). All statistical tests had a significance level of $P < 0.05$ and were conducted using GraphPad Prism Version 10.2.3 for Windows (GraphPad Software Inc., San Diego, USA).

3. Results

3.1. Growth Performances

The survival rate of juvenile tiger shrimps *Penaeus monodon* was 100% in all experimental groups. WG of shrimps fed P2 was significantly higher than that of those fed the control diet. However, there were no significant differences in WG of shrimps fed the FHE-supplemented diets. Similar case was also found for ADG parameter. However, the highest LG was significantly observed when shrimps were fed with VM2 and VM3. Growth performances of the tiger shrimp during the feeding trial is presented in Table 3.

Table 3. Growth performances of tiger shrimp *P. monodon* fed the experimental diets.

Treatment	Growth indicators		
	WG (g)	ADG (%)	LG (cm)
P0	1.59 \pm 0.24 ^a	2.42 \pm 0.29 ^a	2.16 \pm 0.06 ^a
P1	1.93 \pm 0.21 ^{ab}	3.03 \pm 0.19 ^{ab}	2.27 \pm 0.03 ^{ab}
P2	2.62 \pm 0.09 ^b	3.78 \pm 0.39 ^b	2.55 \pm 0.17 ^b
P3	2.24 \pm 0.07 ^{ab}	3.10 \pm 0.08 ^{ab}	2.54 \pm 0.05 ^{ab}
P4	2.02 \pm 0.37 ^{ab}	2.83 \pm 0.38 ^{ab}	2.25 \pm 0.04 ^{ab}

Data are presented as the mean \pm SE of three replicates. Values in the same column with the same superscript are not significantly different ($P > 0.05$). WG: weight gain, ADG: average daily gain, LG: length gain

3.2. Moulting Performances

Throughout the feeding experiment, moulting performance (MP) of the shrimps was significantly higher in those fed P2 and P3 than in other diets. However, the MP of shrimps fed P3 was not significantly different than those fed P0, P1, and P4 (Figure 1).

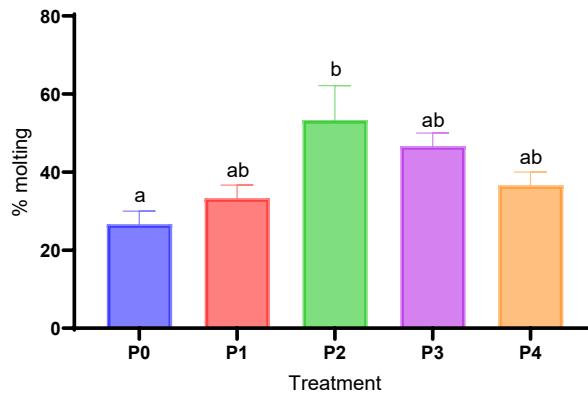


Figure 1. Moulting performances of tiger shrimp *P. monodon* fed the experimental diets.

3.3. Feed Efficiency

With respect to feed efficiency (FE), significant difference was noticed among treatments after 30 days. Supplementation of fermented herbal extract (FHE) had significant effect on FE level: P2 application showed better result in improving the FE of juvenile tiger shrimp *P. monodon* (Figure 2).

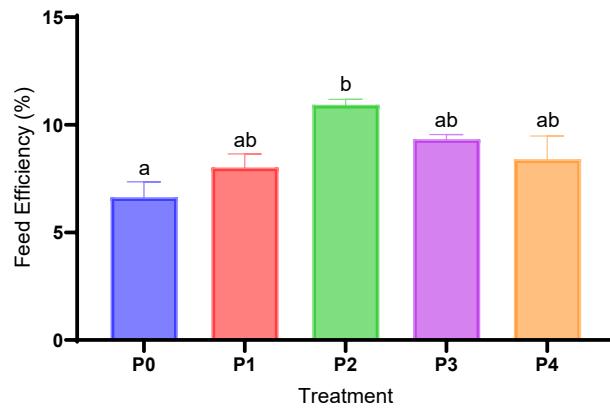


Figure 2. Feed efficiency of tiger shrimp *P. monodon* fed the experimental diets.

3.4. Nutrient Retention

In Figure 3, the P2 treatment group showed significantly higher protein retention (PR) compared to P0, although there were no significant difference in PR among P2, P1, P3 and P4 treatment groups.

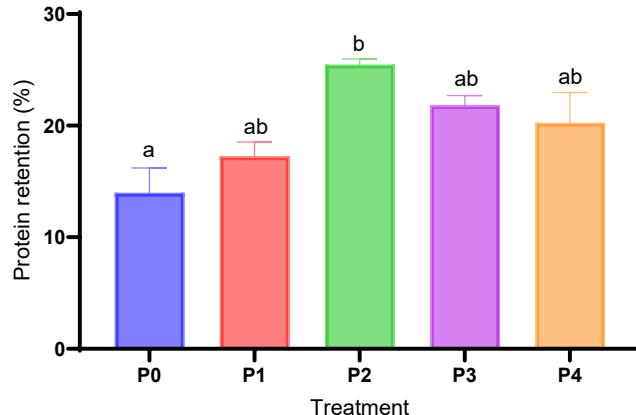


Figure 3. Protein retention of tiger shrimp *P. monodon* fed the experimental diets.

On the other hand, the supplementation of herbal extract (FHE) had no significant effect on the lipid retention (LR) of the juvenile *P. monodon*, as shown in Figure 4. The LR of the experimental animals ranged from 2.85 % to 4.15 % among all treatment groups.

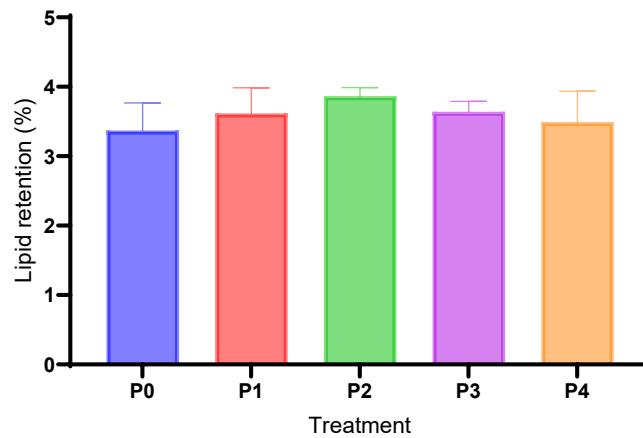


Figure 4. Lipid retention of tiger shrimp *P. monodon* fed the experimental diets.

As presented in Figure 5, the energy retention (ER) of juvenile *P. monodon* supplemented with 100 ml/kg fermented herbal extract (P2) was significantly higher compared to all other supplementation groups. The lowest value in ER was observed when diets were not supplemented with the herbal extract.

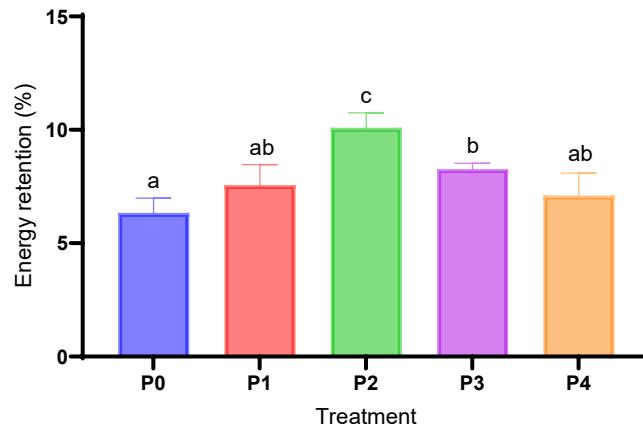


Figure 5. Energy retention of tiger shrimp *P. monodon* fed the experimental diets.

3.5. Proximal Composition of Shrimp's Flesh

The proximal composition of juvenile tiger shrimp is described in Table 4. Crude protein (CP) was the lowest in the control group (70.56%), and juvenile tiger shrimp fed P2 and P3 diets attained the highest CP content among all treatment groups. Whilst the highest crude lipid (CL) content was found in juvenile tiger shrimp fed the control diet (P0), the lowest crude lipid (CL) content was observed in juvenile tiger shrimp fed P2 and P3 diets. Furthermore, crude fibre (CF) of juvenile tiger shrimps was not significantly different among all treatment groups. No significant difference with regard to nitrogen free extract among all treatment groups. Ash content was higher in the controlled diet, compared to that from other dietary treatments.

Table 4. Proximal composition of tiger shrimp *P. monodon* fed the experimental diets.

Treatment	Composition						
	Moisture (%)	CP (%)	CL (%)	Carbohydrate		Ash (%)	Energy (kcal/g)
P0	81.39 ± 0.52 ^c	70.56 ± 0.18 ^a	4.37 ± 0.07 ^d	1.05 ± 0.07 ^{ab}	1.70 ± 0.03	22.32 ± 0.21 ^b	289.23 ± 2.33 ^a
P1	80.15 ± 0.28 ^{bc}	75.29 ± 0.26 ^b	4.16 ± 0.06 ^c	1.15 ± 0.04 ^b	2.21 ± 0.63	17.19 ± 0.57 ^a	305.61 ± 1.85 ^b
P2	71.88 ± 0.77 ^a	82.37 ± 1.14 ^c	1.98 ± 0.04 ^a	0.75 ± 0.11 ^a	1.04 ± 0.09	18.85 ± 1.05 ^a	308.81 ± 3.06 ^b
P3	77.71 ± 0.78 ^a	77.50 ± 1.14 ^c	2.37 ± 0.03 ^a	0.93 ± 0.09 ^a	1.46 ± 0.22	17.75 ± 1.18 ^a	296.42 ± 2.74 ^a
P4	80.42 ± 1.07 ^b	76.31 ± 0.35 ^b	2.84 ± 0.05 ^b	1.26 ± 0.03 ^{ab}	1.48 ± 0.06	18.12 ± 0.36 ^a	296.94 ± 2.55 ^a

Data are presented as the mean ± SE of three replicates. Values in the same column with the same superscript are not significantly different ($P > 0.05$). CP: crude protein, CL: crude lipid, CF: crude fibre, NFE: nitrogen free extract.

4. Discussion

Over the past few decades, there has been significant research into the use of medicinal herbal plants or their extracts as feed supplements in aquaculture species, including shrimp. These compounds have a long history of being used in fish and shrimp production because of their favorable effects on feed palatability, growth performance, antioxidant capabilities, anti-inflammatory responses, immunological modulation, and resistance against some pathogenic microorganisms [19–30].

To the best of our knowledge, the effects of fermented herbal extracts (FHE) from the three combined plant-based ingredients of mulberry leaf (*M. alba*), Javanese turmeric (*C. xanthorrhiza*), and fingerroot (*B. rotunda*) on growth performances and feed utilization of juvenile tiger shrimp *P. monodon* is first recorded in the present study. Throughout the experiment, dietary supplementation with FHE as phytobiotic improved the growth of juvenile tiger shrimp, as evidenced by better WG, ADG, and LG. Incorporation of FHE at 100 mg/kg improved tiger shrimp WG by approximately 65%, compared to the control diet. Various herbal supplements have undergone testing and demonstrated comparable growth-enhancing properties of shrimp, including seaweeds extract [31], *Macleaya cordata* leave [32], *Panax notoginseng* water extract [33], *Piper betle* and *Phyllanthus emblica* leaf extracts [34], *Diplazium esculentum* extract [35], *Terminalia catappa* L. tree [36], *Astagalus membranaceus* and *Eucommia ulmoides* extract [37], *Bidens alba* and *Plectranthus amboinicus* leaves [38], *Solanum trilobatum*, *Curcuma longa*, *Psidium guajava*, *Ocimum sanctum*, *Azadirachta indica*, *Acalypha fruticosa*, *Centella asiatica*, *Bacopa monnieri*, *P. betle*, and *Leucas aspera* extract [39], *Achyranthes aspera* extract [40], *Citrus lemon* extract [41], *Alpinia officinarum* stems and leaves extract [42], and *Asparagus cochinchinensis* and *P. notoginseng* extract [23].

Moreover, research has demonstrated that the effects of herbal extracts on aquaculture species growth are dose-dependent [43]. Growth typically rises until a dietary extract inclusion level (also known as the optimal inclusion level), after which it falls as extract inclusion levels rise. For example, dietary *Aloe vera* polysaccharides crude powder extracts optimum supplementation level of between 1.76 and 1.79% per kg diet was found most suitable for maximum growth in African catfish (*Clarias gariepinus*) fingerlings [44]. Similarly, 2.0%/kg diet of polyherbal (*S. trilobatum*, *C. longa*, *P. guajava*, *O. sanctum*, *A. indica*, *A. fruticosa*, *C. asiatica*, *B. monnieri*, *P. betle* and *L. aspera*) supplement was recommended to be the dietary optimum level that can enhance growth and immunity in shrimp *Penaeus vannamei* [39]. In addition, the optimum dietary inclusion level of *Achyranthes aspera* extract seemed to be 0.2–0.4 mg/kg diet for juvenile Pacific white shrimp *Penaeus vannamei* [40]. Indeed, it has been noted that higher levels of herbal extract in fish diets might lead to poor or no growth, which is mostly caused by increased concentrations of anti-nutritional components, toxic substances, and excessive doses [45–50].

The current study has demonstrated that FHE contains phytochemical compounds such as sitosterol, alkaloid, saponin, flavonoid, and curcumin. The growth enhancement seen in this study may be linked to the presence of abundant phenolic chemicals and other phytochemicals in FHE described in the present study. Phytochemical substances such as tannin, saponin, flavonoid, steroid,

terpenoids have also been demonstrated to be responsible for enhanced growth and physiological status of *C. gariepenus* fingerlings [51]. In addition, herbal phytochemicals are reported to be biotransformed by gut bacteria, resulting in bioavailable molecules having antimicrobial, anti-inflammatory, antioxidant, and digestive effects [33,36,52–55].

The phytochemical compounds contained in the diets may be responsible for the reported moulting performance (MP) effects of the FHE. The beneficial effect of FHE on MP of tiger shrimp in the present study is consistent with previous investigation suggesting that synchronized molting in crustacean can be enhanced by phytoecdysone, which act as a stimulant [35]. Shrimp, like all other arthropods, grow by first untying the connectives that separate the living mass from the exoskeleton, or extracellular cover, and then rapidly letting it absorb water to expand its new elastic cuticle and harden it quickly for defense, feeding, movement, and body structure [56]. Moulting is a periodic process in crustaceans where they shed their exoskeleton as part of their growth. Shrimp growth is a non-continuous process that is controlled by the moult cycle. This cycle consists of brief periods of rapid development during moulting, and longer intervals between moults where no growth takes place [57]. The behavioural and metabolic adaptations that occur during the shrimp moulting cycle have important implications for energy balance and feed utilization in aquaculture settings [58]. In aquatic organisms, growth takes place when the nutrients absorbed from diets surpass the requirements for basal energy and activity [59]. Based on the findings, it can be suggested that FHE has the potential to enhance the feed efficiency (FE) of juvenile tiger shrimp. This is likely due to the ability of FHE to facilitate the digestion and absorption of nutrients in tiger shrimp, leading to improved growth.

The primary digestive enzymes found in the intestines of fish include amylase, lipase, and protease. Assessing the activity of digestive enzymes in the intestines of fish is a crucial indicator that reveals the physiological function of fish. This measurement helps determine how well fish absorb and utilize nutrients [27,28,60]. Supplementation of herbal extract as feed additive enhanced the excretion of protease, lipase, and amylase enzymes in the intestines of fish, resulting in enhanced nutrient absorption and improved fish growth [54,61]. The bioactive chemicals present in seed powder have the potential to improve intestinal epithelial cell function and growth performance by inducing the release of digesting enzymes. Consequently, this can promote the viability of advantageous gut microorganisms and optimize the assimilation of absorbed nutrients. In the present study, the oral administration of FHE improved protein absorption efficiency and energy retention in juvenile tiger shrimp, which might be due to increased secretions from the digestive system, as reported by several studies [27,33,62].

The dietary requirements and developmental potential of fish have a direct impact on their body composition. The nutritional quality of fish is frequently assessed by examining the total crude fat and crude protein content of the entire body. The study discovered that fish that were fed a diet supplemented with FHE at 100 mg/kg or 150 mg/kg feed had the maximum whole-body protein content, indicating an increase in protein retention efficiency, which could also be associated with the improvement of FE in FHE-treated groups. The substantial protein content of the FHE, which has been reported to be comparable to that of other protein-rich plants such as soy, lupin, and pea, may be responsible for the increase in protein content. It was notable that while muscle CL contents were significantly upregulated by FHE treatment, whole-body CL contents in the FHE-supplemented groups were lowered, following a trend likely opposite to that of whole-body CP. This showed great capabilities of phytobiotic on gaining healthier shrimp body composition and higher nutritional value for human consumers, as suggested by previous research [63]. Further, compared to the control group, the increased energy content in the whole-body proximal composition of the experimental animals fed with FHE-supplemented diets at 50 mg/kg or 100 mg/kg feed suggests a positive effects of FHE on feed utilization throughout the rearing period.

It was discovered that fermentation works well to break down ANFs, crude fiber, and carbohydrates, as well as to increase the amount of crude protein and peptides and improve the amino acid composition of plant sources [12,64]. In addition to enhancing nutritional value, fermentation has been shown to boost the feed's synthesis of antioxidants such as flavonoids,

phenolics, and g-aminobutyric acid [13,65]. Thus, once the herbs undergo fermentation with probiotics, the abundant probiotics can enhance the efficiency of feed utilization in the animal's body and stimulate the metabolism of nutrients, hence enhancing the animal's production performance. While *Lactobacillus* plays an important role in the sensory, nutritional, and hygiene of various fermented products, as well as producing a variety of aromatic compounds that impart flavor, *Saccharomyces* (yeast) is a eukaryotic single-cell microorganism that can convert carbohydrates into carbon dioxide and alcohol, and is frequently used in biomass production due to its ability to rapidly utilize a variety of carbon sources and produce large amounts of protein [13]. The influences of fermented herbal products on growth and feed utilisation of cultured fish and shrimp have been well documented by several studies. For instance, it was emphasized that when grouper were fed diets supplemented with dried lemon peel herb, the growth performance was deteriorated, however biotechnological approach through fermentation of the ingredient with *Lactobacillus plantarum* had no adverse impact on fish growth [66]. Therefore, better growth and feed utilization of juvenile tiger shrimp fed FHE-supplemented diets as demonstrated in the current study can be associated with the advantageous roles of fermentation approach.

Phytobiotic supplementation offer efficient and environmentally-friendly methods for treating fish ailments while ensuring the preservation of the environment's sustainability. For example, it is documented that the presence of polyphenolic flavonoids, which chelate free radicals, can have a protective effect against the peroxidative damage that can occur in the liver and kidney [53]. Herbal extracts can serve as effective natural substitutes for antibiotics or chemical medications in the treatment of black tiger shrimp *P. monodon* against pathogenic *Vibrio parahaemolyticus* [34]. They can also help mitigate issues related to drug resistance and the accumulation of residues in fish and consumers [67].

5. Conclusions

The present study documented that the supplementation of FHE through top-coating technique improved the growth, feed efficiency, nutrient retention, and crude protein whole body content of *P. monodon*. These factors are crucial for the successful application of aquaculture practices. Based on the findings, it is recommended that the juvenile tiger shrimp's diet should contain 100 mg/kg of FHE for optimal results. Thus, the current study demonstrates that employing FHE as a phytobiotic is beneficial for increasing aquaculture productivity in tiger shrimp, which could contribute to the sustainable growth of the aquaculture industry in the future.

Author Contributions: Conceptualization, I.I. and Y.F.; methodology, Y.F.; software, I.I. and S.S.; validation, I.I. and S.S.; formal analysis, I.I.; investigation, S.S.; resources, Y.F and S.S.; data curation, S.S.; writing—original draft preparation, I.I.; writing—review and editing, Y.F.; visualization, S.S.; supervision, Y.F.; project administration, I.I.; funding acquisition, I.I. and S.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Center for Education, Indonesian Ministry of Marine Affairs and Fisheries. The APC was funded by Marine and Fisheries Polytechnic of Jembrana (Bali, Indonesia).

Institutional Review Board Statement: All animal care and experimental methods used in this study were authorized by the Jakarta Technical University of Fisheries' Institutional Animal Care and Use Committees (Jakarta, Indonesia).

Data Availability Statement: Upon reasonable request, the datasets of this study can be available from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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