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The Effect of Feed Supplementation with Fermented Red Seaweed (*Kappaphycus alvarezii*) on Growth and Survival of Whiteleg Shrimp (*Litopenaeus vannamei*) Post-Larvae Culture

Gede Suantika[1], Magdalena Lenny Situmorang[1*], Felicia Irene Saputra[1], Ulya Alviredieta[1], Pingkan Aditiawati[1], Sastia Prama Putri[1, 2]

[1]Microbial Biotechnology Research Group, School of Life Sciences and Technology, Institut Teknologi Bandung, Bandung, Indonesia
[2]Department of Advanced Science and Biotechnology, Osaka University, Osaka, Japan

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ABSTRACT

This study was aimed to investigate the effect of novel shrimp diet supplemention with fermented red seaweed *Kappaphycus alvarezii* on growth and survival of whiteleg shrimp (*Litopenaeus vannamei*) post-larvae culture. The research consisted of three consecutive steps: (1) preliminary study on *Kappaphycus alvarezii* fermentation, (2) feed formulation and proximate analysis, (3) performance test of commercial feed (C), commercial + 0.5% *K. alvarezii* (EF1), and commercial + 1.5% fermented *K. alvarezii* (EF2). Following 15 days of feeding, the highest biomass, specific growth rate and survival were achieved in EF2 treatment (1.60±0.21 g, 10.88±0.86%/d, 91.67±1.53%), significantly different from control (0.99±0.09 g, 7.72±0.63%/d, 47.00±5.00%) ($p<0.05$). Overall, this study suggested that the dietary supplementation of 1.5% fermented *K. alvarezii* can enhance the growth and survival of whiteleg shrimp during the nursery phase.

1. Introduction

Shrimp production worldwide is expected to grow by more than 5.6% annually. In 2017, the global market for shrimp, including farm-raised (aquaculture) and wild-caught shrimp, was valued at around $40 billion (FAO 2020). The dominant species of farmed shrimp, *Litopenaeus vannamei*, or whiteleg shrimp, accounts for about $14 billion alone. The Indonesian aquaculture shrimp industry is currently in a strong competitive position in the global market, with an expected growth of 8% per year through 2022, surpassing global growth rates of 5.6% (Rubel *et al.* 2019).

Unfortunately, white shrimps are vulnerable to environmental stress and *Vibrio harveyi* as pathogen during hatchery or nursery phase. Vibriosis disease leads shrimps into massive mortality and disserves aquaculture industrial economy (Liu *et al.* 2004) Several strategies such as application of recirculating aquaculture system (RAS) technology (Suantika *et al.* 2003), zero water discharge (ZWD) (Suantika *et al.* 2015), hybrid ZWD-RAS (Suantika *et al.* 2018), as well as probiotic, prebiotic and/or synbiotic administration through shrimp feed (Suantika *et al.* 2013; Situmorang *et al.* 2020, 2021; Uawisetwathana *et al.* 2021) have been developed to prevent *Vibrio* dominancy and distribution on shrimp culture by controlling the water quality and microbial community itself. Along with system improvement, resistance against vibriosis syndrome could be increased through the manipulation of shrimp feed. Several studies are focused on the use of seaweed products and byproducts as immunostimulant agent in shrimps (Chojnacka *et al.* 2012; Sivagnanavelmurugan *et al.* 2014). Even though the use of seaweed is proven in enhancing shrimp resistance against vibriosis due to its polysaccharide properties (Chojnacka *et al.* 2012; Marudhupandi and Inbakandan 2015), in many cases the effect of seaweed application and supplementations contribute in lowering the growth and uncertainty of immune response. Based on this situation, an effort to enrich the nutritional properties of seaweed responsible for immunity improvement such as the availability of essential amino acids and fatty acids should be taken as a serious consideration. One alternative strategy to overcome this consideration

* Corresponding Author
E-mail Address: situmorangml@sith.itb.ac.id

is nutrition improvement of seaweed products by fermentation (Han *et al.* 2012). Several microbial fermentation agents which has been known due to its ability improving the amino acid profile of plant-based raw material, including *Lactobacillus brevis*, *L. casei* (Uchida and Miyoshi 2013), *L. plantarum*, *L. acidophilus* (Uchida and Miyoshi 2013; Swain *et al.* 2014), *Leuconostoc mesenteroides, Pediococcus pentosaceus, Weisella confuse, Tetragenococcus halophilus* (Uchida and Miyoshi 2013; Nuraida 2015), and *Saccharomyces cerevisiae* which has ability to enhance amino acid profile of red seaweed *Kappaphycus alvarezii* (Ferreira *et al.* 2010).

This research was aimed to evaluate the effect of shrimp feed supplementation with fermented *K. alvarezii* at the concentration of 0.5% and 1.5% compared to the commercial feed as control treatment on the growth and survival of white shrimp post-larvae culture.

2. Materials and Methods

2.1. Preparation and Fermentation of *Kappaphycus alvarezii*

2.1.1. Source of *K. alvarezii*

The raw *K. alvarezii* were obtained from seaweed farm in Bali and provided by PT. Gapura Akua Kultiva, Bandung, Indonesia.

2.1.2. Rinsing and Milling of *K. alvarezii*

K. alvarezii were washed three times with freshwater to remove debris and salt residues, chopped (±5 cm), and dried at 60-75°C for 24-48 h. Dried *K. alvarezii* were further milled using 250 µm disc mill to obtain powder.

2.1.3. Fermentation of *K. alvarezii*

Fermentation of *K. alvarezii* was done using *S. cerevisiae* at 25°C, 125 rpm. Fermentation medium was prepared using 20 g *K. alvarezii* powder, 1.5 g corn starch powder, 0.4 g urea, 1 g glucose, solved in 200 ml aquadest. The activation and adaptation of *S. cerevisiae* was done to build the capacity of *S. cerevisiae* to use *K. alvarezii* substrate (Asaduzzaman 2007), following the consecutive steps: (1) activation of *S. cerevisiae* in 100% premix medium (potato dextrose broth or PDB), (2) adaptation of *S. cerevisiae* in 25% *K. alvarezii* medium and 75% premix medium, (3) adaptation of *S. cerevisiae* in 50% *K. alvarezii* medium and 50% premix medium, (4) adaptation of *S. cerevisiae* in 75% *K. alvarezii* medium and 25% premix medium, and (5) activation of *S. cerevisiae* in 100% *K. alvarezii* medium. Following the activation and adaptation of *S. cerevisiae* in *K. alvarezii* medium, the *K. alvarezii* medium was fermented for 72 h by 10% active-*S. cerevisiae*. The cell density of *S. cerevisiae* in 100% *K. alvarezii* medium was measured using haemocyte count, while pH value was measured every six hour to plot the growth curve (Asaduzzaman 2007). The fermentation was stopped after 72 h and the fermented *K. alvarezii* medium was oven dried at 60-75°C for 24-48 h and further milled using 250 µm disc mill to obtain fermented *K. alvarezii* powder.

2.2. Proximate Analysis of Raw and Fermented *K. alvarezii*

Proximate analyzes were done on five hundred gram of each raw and fermented *K. alvarezii* (RM1 and RM2 respectively) at PT. Saraswanti Indo Genetech, Bogor, Indonesia, to measure the total energy, energy from fat, dry matter, water content, ash content, total lipid, total protein, total carbohydrate and fiber, amino acid profile and fatty acid profile (AOAC 1999).

2.3. Experimental Feeding

2.3.1. System Set Up

The sea water in tank was sterilized using 60 ppm chlorine and aerated for 24 h. After 24 h, water was neutralized by Na-thiosulphate (1:1). Following sterilization, the three days-acclimated *L. vannamei* (PL-13) were transferred to 70 l hatchery tanks containing sea water at 30 ppt, 28°C, pH 8, and DO 8.0 mg/l, at the stocking density of three individuals per liter (APHA 1999).

2.3.2. Feeding Regime

Three types of feed were tested in this study: (1) commercial feed as control (C), (2) commercial feed supplemented with 0.5% *K. alvarezii* (EF1), and (3) commercial feed supplemented with 1.5% fermented *K. alvarezii* (EF2). Daily feeding was done in three portions (8 AM, 1 PM and 8 PM) at the rate of 10% body weight during 15 days experiment (T0-T14). The following illustration shows the experimental layout during the feed trial. Each type of feed was tested in three replicates (triplo), which showed by the different numbers.

2.3.3. Water Quality Measurement

The value of water quality physical-chemical parameters were controlled within the tolerance range and examined per two days during 15 days feed performance test. The water removal daily is 2.5-5.0% per day to maintain the salinity in case of evaporation. The maintenance of physical-chemical parameters were required to reduce the experimental variables and truly focus on effect of fermented *K. alvarezii* treatment. The dissolved oxygen and water temperature were consecutively maintained approximately at 8.0 mg/l, 28°C and measured utilizing Hach®40qd with 0.005 mg/l and 0.05°C. pH value was maintained at 8.0 measured with Mettler-Toledo®, 0.005 accuracy. Water salinity was measured using ATAGO® refractometer, 0.5 ppt and maintained at 30 ppt. Ammonium, nitrite and nitrate concentration were measured with Sera® kit and maintained within the tolerance range (Wyban *et al.* 1995; Abowei 2010).

2.3.4. Biological Analyses

Twenty shrimp were sampled randomly from each replicate tank at the initial (T0), middle (T7) and final (T14) period of feeding test for biological analyses. Shrimp length was measured using scientific ruler. Shrimp weight was measured on analytical scale to calculate specific growth rate (SG), mean body weight (MBW) and biomass value. Besides growth performance value, survival of shrimp PL was calculated as important aim of this research. The equation to calculate the growth performance parameter and survival were shown below (Suantika *et al.* 2012):

$$SG = \frac{(\ln W_t - \ln W_o)}{t} \times 100\%$$

$$MBW = \frac{Wshrimp}{n}$$

$$Biomass = Shrimp\ population \times MBW$$

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

Where:
W_t = final weight of *L. vannamei* PL
W_o = initial weight of *L. vannamei* PL
t = duration of experiment
n = amount of *L. vannamei* weighed
N_t = amount of shrimp on T14
No = amount of shrimp on T0

The food conversion ratio (FCR) and feed efficiency ratio (FER) were calculated using the following formula (Wyban *et al.* 1995):

$$FCR = \frac{Amount\ of\ feed}{(W_t - W_o)}$$

$$FER = \frac{(W_t - W_o)}{Amount\ of\ feed}$$

Where:
W_t = final weight of *L. vannamei* PL
W_o = initial weight of *L. vannamei* PL

2.3.5. Statistical Analysis

Survival data were first normalized using an arcsine transformation before statistical analysis (Situmorang *et al.* 2021). To evaluate the differences between treatment groups, data on growth and survival parameters were analyzed using one-way ANOVA and followed by Duncan posthoc Test with 95% confidence intervals. All statistical analyses were performed using SPSS® Version 24.0.

3. Results

3.1. Proximate Analysis

The result of proximate analysis on *K. alvarezii* as feed ingredient is shown in Table 1. It was found that the total protein in fermented *K. alvarezii* (RM2) was three times higher compared to raw *K. alvarezii* (RM1) itself. The amino acid (Table 2) and fatty acid (Table 3) content in RM2 were also found to be higher than RM1, except for tryptophane (zero value) in both RMs. Table 2 shows the escalation of the amino acid contents of the fermented *K. alvarezii* (RM2) and the feed with RM2 supplementation compared to other experimental feeds.

According to the feed proximate result, the general proximate (Table 1) and fatty acid profile (Table 3) for commercial feed supplemented with 0.5% *K. alvarezii* (EF1) and commercial feed supplemented with 1.5% fermented *K. alvarezii* (EF2) have had similar value, relatively similar to the nutritional value of the commercial feed (C).

3.2. Shrimp Growth and Survival Performance

The feeding performance test was performed to verify the effect of feed supplementation with fermented K. alvarezii on the growth and survival of shrimp post-larvae. During the test, physical and chemical water quality parameters were maintained at the tolerance level for shrimp PL (Table 4).

Table 1. Proximate of *K. alvarezii* as feed ingredients and different experimental feeds

Proximate component	Unit	Nutrition value				
		Proximate component		As feed		
		RM1	RM2	C	EF1	EF2
Total energy	kcal/100 g	288.38	308.81	2926.7	2913.4	2887.3
Energy from fat		1.98	2.61	37.2	37.0	36.7
Dry matter	%	91.04	97.33	79.0	88.9	89.0
Water content		8.96	2.67	9.9	0	0
Ash content		19.22	20.49	11.0	11.0	11.1
Total lipid		0.22	0.29	4.1	4.1	4.1
Total protein		3.26	9.13	27.5	27.4	27.2
Total carbohydrate		68.34	67.42	36.4	36.5	36.8
Fiber		4.6	6.78	0	0	0.1

RM1 = *K. alvarezii*, RM2 = fermented *K. alvarezii*, C = commercial feed, EF1 = commercial feed + 0.5% *K. alvarezii*, EF2 = commercial feed + 1.5% *K. alvarezii*

Table 2. Amino acid profile of raw materials of feed and feed variants

Amino acid	Unit	Nutrition value					
		As raw materials		As feed			
		RM1	RM2	C	EF1	EF2	Reference (Velasco *et al.* 2000)
L-Histidine*	g/kg	0.213	0.362	7.573	8.510	12.742	4.00
L-Threonine*		1.532	1.826	13.947	20.851	40.403	7.30
L-Valine*		1.557	2.000	13.654	20.672	42.647	8.60
L-Isoleusine*		1.189	1.461	6.142	8.403	13.944	8.20
L-Leusine*		2.196	2.657	12.062	17.416	33.211	13.80
L-Phenylalanine*		1.305	1.607	23.094	32.974	61.535	8.60
L-Arginine*		1.511	1.580	16.916	22.770	40.116	13.00
L-Glycine		1.566	2.011	20.125	26.464	38.552	8.30
L-Alanine		1.839	2.433	0	6.879	23.062	8.20
L-Aspartic Acid		2.941	3.779	0	7.129	29.358	21.50
L-Glutamic Acid		3.512	5.091	0	8.369	35.519	26.70
L-Proline		1.285	1.602	0	13.390	55.177	9.40
L-Serine		1.487	1.768	0	15.986	74.333	9.00
L-Tyrosine		0.693	0.684	0	5.848	23.396	4.00
L-Lysine*		1.415	1.283	0	6.768	25.810	12.30
L-Methionine*		0.503	0.541	0	3.154	9.986	5.40
L-Trytophan		0	0	1.662	1.654	1.637	3.16

RM1 = *K. alvarezii*, RM2 = fermented *K. alvarezii*, C = commercial feed, EF1 = commercial feed + 0.5% *K. alvarezii*, EF2 = commercial feed + 1.5% *K. alvarezii;* *essential amino acids

4. Discussion

4.1. Proximate Analysis of *K. alvarezii* as Feed Ingredients

Based on the result of proximate analysis on *K. alvarezii* as feed ingredients (Table 1), it is known that total protein in fermented *K. alvarezii* (RM2) was increased three times compared to raw *K. alvarezii* (RM1) itself. This is potentially due to the character of *S. cerevisiae* as fermentation bio-agent, which is high in protein of up to 40% dry matter (Yamada and Sgarbieri 2005). The purpose of raw materials supplementation in shrimp commercial feed are: (1) to fulfill the nutrition value of shrimp diet, (2) to add essential nutrients for shrimp growth such as amino acid and fatty acid of the diet, and (3) to maximize the cost-effectiveness of diet production. As mentioned earlier, *S. cerevisiae* may enhance the amino acid contents in feed (Yamada and Sgarbieri 2005). Table 2 shows the escalation of the amino acid contents of the fermented *K. alvarezii* (RM2) and the feed with RM2 supplementation compared to other

Table 3. Fatty acid profile of raw materials of feed and feed variants

Fatty acid		Unit	Nutrition value				
			As raw materials		As feed		
			RM1	RM2	C	EF1	EF2
Myristoleic acid	C 14:0	%	0.213	0.362	2.498	2.485	2.460
Palmitate acid	C 16:0		1.532	1.826	8.889	8.845	8.758
Palmitoleic acid	C 16:1		1.557	2.000	2.542	2.530	2.504
Stearic acid	C 18:0		1.189	1.461	2.213	2.202	2.180
Oleic acid	C 18:1 ω9C		2.196	2.657	6.311	6.280	6.217
Linoleic acid	C 18:2 ω6C		1.305	1.607	8.613	8.570	8.485
Linolenic acid ω3	C 18:3 ω3		1.511	1.580	0.978	0.973	0.963
Arachidonic acid	C 20:4 ω6		1.566	2.011	0.551	0.548	0.543
Omega-6			1.839	2.433	9.289	9.243	9.150
Omega-9			2.941	3.779	6.480	6.448	6.384
Unsaturated lipid			3.512	5.091	26.685	26.552	26.286
Saturated lipid			1.285	1.602	14.649	14.577	14.432
MUFA			1.487	1.768	9.911	9.862	9.763
PUFA			0.693	0.684	16.774	16.690	16.522

RM1 = *K. alvarezii*, RM2 = fermented *K. alvarezii*, C = commercial feed, EF1 = commercial feed + 0.5% *K. alvarezii*, EF2 = commercial feed + 1.5% *K. alvarezii*

Table 4. Physico-chemical water quality parameters during 15 days experimental period

Parameter	Unit	Parameter	Tolerance range
Salinity	ppt	32–41	5–40
Temperature	°C	28–30	28–32
DO	mg/l	7.3–9.0	>3.0
pH		7.5–8.5	7.5–9.0
Ammonium	ppm	0.1–0.4	<3.9
Nitrite	ppm	0–0.7	≤5
Nitrate	ppm	0–10	≤60

experimental feeds. The amino acid (Table 2) and fatty acid (Table 3) content in RM2 are evenly higher than RM1, except for tryptophane (zero value) in both RMs. This implies that fermentation on *K. alvarezii* by 10% *S. cerevisiae* with six hours-inoculum age on *K. alvarezii* medium allegedly increases protein, amino acid and some fatty acid nutritions compared to *K. alvarezii* raw material itself.

EF1 and EF2 provide essential fatty acid needed by *Litopenaeus vannamei* (Han *et al.* 2012), such as linoleic acid (ω6), linolenic acid (ω3), EPA (C 20:5 ω3), DHA (C 22:6 ω3), and poly-unsaturated fatty acid (PUFA), which may enhance health of shrimp, acts as antioxidant and anti-neoplasm in shrimp (Chojnacka *et al.* 2012; Dayal *et al.* 2013).

Protein, the important macromolecule to furnish a continuous supply of essential amino acids, influences the growth rate or size of the shrimp. The protein requirement is minimally reported as 25% of the diet (Davis 2005). In this study, the amino acid profile on C, EF1 and EF2 feed were also analyzed. The amino acid value of EF1 and EF2 feeds both exceeded the C feed. Alanine, aspartic acid, glutamic acid, proline, serine, tyrosine, lysine and methionine, were contained in EF1 feed and highest in EF2 feed, which at first were not available in C feed (Table 2). The highest amino acids was obtained from EF2 feed, up two to three times higher than C feed. The amino acid profile of EF2 meet the shrimp requirement based on Velasco *et al.* (2000) and even higher than the reference. The increase in essential amino acid and non-essential amino acid suits the shrimp basic and additional requirement. Those amino acids have important roles in maintaining the growth and survivalof *L. vannamei* PL during nursery. Increment on branched chain amino acid (BCAA), such as isoleusine, valine, and lysine play role as growth promoter on aquaculture organisms (Rahimnejad and Lee 2014). The alanine, glycine and proline are known as osmolytes which participate in intracellular osmoregulation (Lemme 2010), the free amino acids help to maintain SOD capacity and suppress reactive oxygen intermediate (ROIs) inside shrimp regulation system (Li *et al.* 2015). This suppression will enhance *L. vannamei* PL tolerance against environmental stress, especially during oxidative stress. Three fundamental components of serine protease, namely serine, proline, and histidine were all found rather high in EF2 feed, of 74.3 g/kg, 55.2 g/kg and 12.7 g/kg, respectively. Serine protease is protein which activate pro-PO to PO enzyme as innate immunity response in penaeids (Song and Li 2014). This enzymatic conversion will trigger the emerging of phenol hydroxylation to yield melanine compound, encasing the pathogenic bacteria or

foreign substances inside shrimp's body (Guzman *et al.* 2009). It is one of several mechanisms to enhance innate or non-specific immunity in shrimp and be more resistant against pathogenic bacteria.

4.2. Shrimp Growth and Survival Performance

All physical and chemical water quality parameters were maintained at the tolerance level for shrimp during the experimental period (Lazur 2007; Timmons and Ebeling 2007; Boyd and Tucker 2014). Following 15 days of feeding test, it was shown that both EF1 and EF2 feed can significantly increase the specific growth rate, total biomass and survival of the shrimp when compared to the control feed. The highest specific growth rate, total biomass and survival was found in EF2 feed of 10.88±0.86%/d, 1.60±0.21 g and 91.67±1.53%, respectively. The use of supplemented feed with fermented *K. alvarezii* can significantly increase the shrimp survival of up to 45% when compared to control feed (Table 5). This could be influenced by the high essential amino acids content (Table 2), including BCAA, osmolyte-role free amino acids, and even proteins or enzymes built from amino acid contents (Song and Li 2014) in the EF2 feed, which are suggested by few studies to be able to enhance the growth and survival of shrimp against environmental stress or pathogens (Dayal *et al.* 2013).

5. Conclusion

According to the result in this research to know the effect of fermented *Kappaphycus alvarezii* feed supplementation on *Litopenaeus vannamei* post-larvae growth and survival, it has been proved that the fermented *Kappaphycus alvarezii* feed supplementation improved white shrimp post-larvae growth performance and survival significantly compared to commercial feed-treatment. This is estimated being caused by the value of essential amino acid content (threonine, leusine, valine, isoleusin, histidine, phenylalanine, arginine, lysine, methionine) and value of non-essential amino acid content (alanine, aspartic acid, glutamic acid, proline, serine and tyrosine), which serve as important protein's building block, growth promoter and survival enhancer, are found higher in fermented *K. alvarezii* supplemented feed rather than commercial feed.

Further examination on the metabolic profile of the shrimp fed with fermented *K. alvarezii* supplemented feed should be performed in order to evaluate the mode of actions of the substrate in the growth and survival improvement in shrimp culture as well as to evaluate the overall quality improvement of the produced shrimp (Suantika *et al.* 2020; Putri *et al.* 2021). Additionally, feeding test should also be performed in the grow out phase, in order to gain a more complete knowledge on the effect of the fermented *K. alvarezii* supplemented feed in all the shrimp life cycle.

Table 5. Growth performance and survival result during 15 days experimental period

Parameters	Unit	Treatment		
		C	EF1	EF2
Length	mm	15.10±1.3[a]	16.30±0.23[a]	16.10±0.40[a]
SGR	%/day	7.72±0.63[a]	10.74±0.81[b]	10.88±0.86[b]
MBW	gram	0.021±0.004[a]	0.022±0.002[a]	0.017±0.003[a]
Biomass	gram	0.99±0.092[a]	1.56±0.19[b]	1.60±0.21[b]
Survival	%	47.00±5.00[a]	70.00±3.00[b]	91.67±1.53[c]

Means within the same row followed by the same letters are not significantly different (p>0.05)

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