The utilization of black soldier fly larvae meal as a substitution of fish meal in diet for white shrimp, *Litopenaeus vannamei*, grow-out

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Abstract. Black soldier fly larvae (BSFL) meal is an environmentally friendly protein source due to its bioconversion production in organic waste processing and has it has potential as an alternative protein source for shrimp diet. Therefore, this study aimed to evaluate the utilization of BSFL meal as a substitution for fish meal in diet on the growth performance of white shrimp, *Litopenaeus vannamei*. The tested treatment were inclusion levels of BSF L meal namely 0%, 7.5%, 15%, and 22.5% to substitute fish meal in diet. The feeding experiment conducted using 12 of 50 L aquaria, and each equipped with gentle aeration. The white shrimp juveniles with initial weight of 1.20 ± 0.07 g were stocked at a density of 10 shrimp to every aquarium. The test diets were given as much as 8-3% BW/day (decreasing based on shrimp weight development) for 49 cultivation days. The results showed no significant difference between treatments (P>0.05) on the specific growth rate, weight gain, survival rate, feed efficiency, and protein efficiency ratio of white shrimp. In conclusion, the BSFL meal could be used up to the level of 22.5% to substitute fish meal in the diet for white shrimp grow-out.

1. Introduction

Feed is a production factor that greatly contributes to the total production cost of fish and shrimp farming [1,2]. Therefore, the price of feed must be reduced and the efficiency level of its use must be increased. According to Cummins *et al.* [2] more than 90% of shrimp farmers use high protein feed with fish meal as the main protein source. Fish meal is used as the main protein source for fish and crustaceans feed due to its high protein content, excellent amino acid and fatty acid profile, high palatability and digestibility [3–5]. The high need for feed protein has a consequence on the need for fish meal which can reach around 50% in feed formulations [2]. At feed conversion ratio values between 1.5–2, it is required 0.75-1 kg of fish meal or the equivalent of 3-4 kg of fresh trash fish (75% moisture content) to produce one kilogram of shrimp. This fact is considered inefficient and has been criticized by environmentalists. The increasing need for fish meal has triggered concerns that the availability of fish resources (trash fish) in the wild will decrease due to the increasingly uncontrolled level of exploitation. Therefore, it is necessary to have an alternative protein source that can substitute the use of fish meal in shrimp feed so that the activity can become more environmentally friendly.

The potential use of insects as a protein source in feed has been reported by several researchers in the world [2,6–8]. According to Van Huis [9], protein from insects was more economical, environmentally friendly and has an natural important role. It was reported that the insect meal has...
high feed conversion efficiency, can be maintained and produced massively. In addition, insect cultivation can reduce organic waste that has the potential to pollute the environment [10]. Another beneficial factor is that insect-based protein sources do not compete with humans so that it is very suitable for use as animal feed ingredients, including for poultry and fish [11].

One of the protein source from insects that can be potentially developed for shrimp feed ingredients is black soldier fly larvae (BSFL, *Hermetia illucens*) meal. The BSFL meal is produced through bioconversion of organic waste. The processing of organic waste using black soldier fly has been developed well and produces quite a lot of meal larvae which can be harvested and used as fish food [12]. Dry BSFL meal contains 40.01-61.4% protein, 13.34-38.9% lipid, 8.0-12.3% crude fiber, and 7.7-19.7% ash [2,13–15].

The BSFL meal has been widely utilized as feed ingredient for both poultry and fish [12,16]. Elwert *et al.* [17] reported that the growth of broilers was relatively the same between those the fed with BSFL meal and those the fed with fish meal. Maurer *et al.* [18] also reported that the substitution of soybean meal with BSFL meal in diet gave relatively the same egg performance, egg weight and feed efficiency in laying hens when compared to standard feed. Stamer *et al.* [19] reported that BSFL meal could replace fish meal by up to 50% in diet for trout grow-out. For yellow catfish grow-out, BSFL meal protein can replace the use of up to 48% fish meal protein in diet without affecting the growth and feed conversion ratio, it even increasing the competence of the fish's immune system [20]. Cummins *et al.* [2] also reported that defatted BSFL meal could be used in rearing white shrimp to below 25% to replace fish meal [2]. However, information about the use of fullfat BSFL meal is still very limited. Therefore, this study aims to evaluate the use of fullfat BSFL meal to substitute the use of fish meal in diet for white shrimp, *Litopenaeus vannamei*, grow-out.

2. Material and methods
This research was carried out in the web laboratorium of Research Institute for Coastal Aquaculture and Fisheries Extension, Maros, South Sulawesi.

2.1 Test diets
The test diets were artificial diets containing about 42% protein, 9% fat, and a total energy of about 4750 kcal / kg which printed in adjusted size to the need of the test animals. The test diets were formulated using BSFL meal to substitute the use of fish meal for white shrimp grow-out. The tested treatments were various inclusion levels of BSFL meal in the diets, namely: 0%, 7.5%, 15%, 22.5% by reducing the use of fish meal. The proximate composition and profile of amino acid and fatty acid of BSFL meal and fish meal are presented in Table 1, 2, and 3.

### Table 1. Proximate analysis of BSFL meal and fish meal used in the test diets (% dry weight).

| Ingredients    | Crude protein | Lipid | Ash | Crude fibre |
|----------------|---------------|-------|-----|------------|
| BSFL meal      | 45.0          | 25.0  | 12.4| 5.6        |
| Fish meal      | 69.3          | 6.9   | 12.2| 1.2        |

### Table 2. Amino acid profile of BSFL meal and fish meal used in the test diets (% dry weight).

| Amino acid profile | BSFL meal | Fish meal |
|--------------------|-----------|-----------|
| *Non essential:*   |           |           |
| Alanine            | 2.71      | 4.49      |
| Aspartic acid      | 3.58      | 8.34      |
| Cystine            | 0.26      | 0.60      |
| Glutamate          | 4.51      | 11.04     |
| Amino Acid | BSFL meal | Fish meal |
|------------|-----------|-----------|
| Glycine    | 1.88      | 3.99      |
| Proline    | 1.78      | 2.71      |
| Serine     | 1.53      | 2.88      |
| Tyrosine   | 2.03      | 2.06      |
| Arginine   | 1.97      | 4.47      |
| Histidine  | 1.08      | 3.28      |
| Isoleusine | 3.67      | 3.01      |
| Leusine    | 4.43      | 5.55      |
| Lysine     | 2.07      | 6.44      |
| Methionine | 0.62      | 1.85      |
| Phenylalanine | 1.50 | 2.93 |
| Threonine  | 1.38      | 3.18      |
| Valine     | 2.03      | 3.51      |
| Total      | 37.03     | 70.33     |

**Table 3.** Fatty acid profile of BSFL meal and fish meal used in the test diets (% lipid).

| Fatty acid profile | BSFL meal | Fish meal |
|--------------------|-----------|-----------|
| Caprilic acid, C8:0| 0.03      | nd        |
| Capric acid, C10:0 | 0.49      | nd        |
| Lauric acid, C12:0 | 19.77     | 0.24      |
| Tridecanoic acid, C13:0 | 0.03 | 0.05     |
| Myristic acid, C14:0 | 6.16 | 3.11     |
| Myristoleic acid, C14:1 | 0.15 | 0.04     |
| Pentadecanoic acid, C15:0 | 0.08 | 0.61     |
| Cis-10-Pentadecanoic acid, C15:1 | 13.90 | nd       |
| Palmitic acid, C16:0 | nd | 19.32     |
| Palmitoleic acid, C16:1 | 2.31 | 3.75     |
| Heptadecanoic acid, C 17:0 | 0.07 | 0.12     |
| Cis-10-Heptadecanoic acid, C17:1 | nd | 0.15     |
| Stearic acid, C 18:0 | 1.85 | 0.02     |
| Elaidic acid, C 18:1n9t | 0.15 | 0.18     |
| Oleic acid, C18:1n9c | 21.06 | 23.76    |
| Linolelaidic acid, C18:2n9t | 0.09 | 0.03     |
| Linoleic acid, C18:2n6e | 9.77 | 3.22     |
| γ-Linoleic acid, C18:3n6 | 0.06 | 0.06     |
| Linolenic acid, C18:3n3 | 0.26 | 0.95     |
| Cis-11-Eicosanoic acid, C20:1 | 0.79 | 1.25     |
| Cis-11,14-Eicosadienoic acid, C20:2 | 0.03 | 0.41     |
| Cis-8,11,14-Eicosatrienoic acid, C20:3n6 | nb | 0.20     |
### Table 4. Ingredients and proximate composition of test diets (% dry matter).

| Ingredients                          | Test diets |
|--------------------------------------|------------|
|                                     | TM0   | TM7.5 | TM15 | TM22.5 |
| Fish meal                            | 36.0  | 31.5  | 27.0 | 22.5   |
| Shrimp head meal                     | 5.0   | 5.0   | 5.0  | 5.0    |
| BSFL meal (fullfat)                  | 0.0   | 7.5   | 15.0 | 22.5   |
| Soybean meal                         | 23.0  | 23.0  | 23.0 | 23.0   |
| Corn meal                            | 21.5  | 20.0  | 18.5 | 17.0   |
| Tapioca meal                         | 5.0   | 5.0   | 5.0  | 5.0    |
| Fish oil                             | 4.5   | 3.0   | 1.5  | 0.0    |
| Lecitine                             | 1.0   | 1.0   | 1.0  | 1.0    |
| Di-calcium phosphate                 | 1.0   | 1.0   | 1.0  | 1.0    |
| Vitamin C                            | 0.1   | 0.1   | 0.1  | 0.1    |
| Vitamin premix<sup>1)</sup>           | 1.9   | 1.9   | 1.9  | 1.9    |
| Mineral premix<sup>2)</sup>           | 1.0   | 1.0   | 1.0  | 1.0    |
| **Proximate analysis:**              |        |       |      |        |
| Protein                              | 42.8  | 42.6  | 42.9 | 42.3   |
| Lipid                                | 8.6   | 9.0   | 9.1  | 9.3    |
| Nitrogen free-extract                | 37.2  | 36.7  | 36.0 | 36.0   |
| Crude fiber                          | 3.4   | 3.4   | 3.4  | 4.0    |
| Ash                                  | 8.0   | 8.3   | 8.7  | 8.4    |
| Gross energy (Kcal/kg)<sup>3)</sup>  | 4756  | 4760  | 4757 | 4742   |

1) Vitamin mix (in 1 kg of diet): Vit. A 57,000 IU; Vit.D3 19,000 IU; Vit.E 142.5 mg; Vit.K3 22.8 mg; Vit B<sub>1</sub> 57 mg; Vit B<sub>2</sub> 85.5 mg; Vit B<sub>12</sub> 57 mg; Vit C 152 mg; Calcium Pentathenate 76 mg; Folic acid 28.5 mg, Biotin 19 mg, Inositol 237.5 mg, Nicotinamide 380 mg.

2) Mineral mix (in 1 kg of diet): Calcium 325 mg; Phosphor 100 mg; Iron 60 mg; Manganese 40 mg; Zinc 73.5; Copper 3 mg; Sodium 1 mg; Cobalt 1 mg; Iodine 0.75 mg; Potassium 0.035 mg.

3) Calculated from the determined crude protein, crude lipid and carbohydrate of the diet using gross energy conversion coefficients of 5.64; 9.44 and 4.11 kcal/g, respectively [21].

### 2.2. Condition of feeding trial

The test animals were white shrimp juvenile with 1.20 ± 0.07 g initial weight and stocked randomly into 12 aquaria (volume of 50 L) at density 10 shrimps/aquarium. The maintenance containers were
equipped with aeration system and recirculating water. The remaining diet and feces were siphoned every day, while 20% of the water was changed every 2 days. The shrimp fed one of the four test diets with three replicates per dietary treatment. The test diets were given as much as 8-3%/day (decreasing based on the development of shrimp weight) four time per day at 07.30, 11.00, 16.30, and 21.00 for 49 days. Aquaria biomass and daily feed rations were adjusted for mortalities, and the amount of diet fed per day was recorded for each aquaria.

The measurement of water quality variables including salinity, temperature, and pH were carried out every 2 days, while \( \text{NH}_3 \), \( \text{NO}_2 \), \( \text{NO}_3 \), alkalinity were measure every week. Also, the total organic matter, total suspended solid, and phosphate were carried out every 2 weeks.

2.3. Sample collection and chemical analysis

Samples of ingredients, diets and the whole body of shrimps were analyzed for proximate, fatty acids and amino acids. Proximate analysis was carried out according to AOAC [22] method. Moisture level was analyzed after drying the samples at 105°C using the oven (Memmert, Germany) until constant weight and crude protein was determined by micro-Kjeldahl procedure. Lipid with gravimetrically determined by extracting petroleum benzene using Soxtherm apparatus. Crude fiber by heating with alternating acid and base washing, and ash by combustion in a muffle furnace (Barnstead Thermolyne, CA, USA) at 550°C for 24 hours. Fatty acid profile was analyzed using Gas Chromatography (Shimadzu 2010 plus, Tokyo, Japan), whereas amino acid analysis was carried out using high-performance liquid chromatography (HPLC) (Shimadzu 20A, Tokyo, Japan).

2.4. Calculation and statistical analysis

Biological responses of shrimp after 49 days feeding trial were evaluated for several parameters including:

- Specific growth rate (SGR) [23]:
  \[
  SGR(\%\text{/day}) = 100 \times \frac{\ln W_e - \ln W_s}{d} 
  \]
  Where ln is the natural logarithm, \( W_e \) and \( W_s \) are the shrimp weight at the end and beginning of the study respectively, and \( d \) is the day number for shrimp culture.

- Weight gain (WG) [24]:
  \[
  WG(\%) = \frac{\text{body weight gain}}{\text{initial body}} \times 100\% 
  \]

- Voluntary feed intake (VFI) [25]:
  \[
  VFI(\%\text{/day}) = \frac{\text{Total dry feed consumed} \times 100}{\text{final weight} + \text{initial weight}} \times \frac{1}{\text{days culture}} 
  \]

- Feed efficiency (FE) and protein efficiency ratio (PER) [26]:
  \[
  FE(\%) = \frac{\text{Wet body weight gain}}{\text{total dry feed consumed}} 
  \]
  \[
  \text{PER} = \frac{\text{body weight gain}}{\text{total feed consumed} \times \text{protein content in diets}} 
  \]

- Survival rate (SR)
  \[
  SR(\%) = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100\% 
  \]

Data on the specific growth rate, weight gain, voluntary feed intake, feed efficiency, protein efficiency ratio, survival rate and whole body chemical composition were analyzed using a one-way analysis of variance (ANOVA). Significant differences (\( P<0.05 \)) were assessed using Tukey’s HSD
test. All the statistical analyses were done using software SPSS version 21. Data on the content of fatty acids and amino acid of the BSFL meal and fish meals were analyzed descriptively.

3. Results and discussion
The amino acid profile of BSFL meal and fish meal that used in this study are presented in Table 2. The amino acid content (essential and non-essential) of BSFL meal was lower than the amino acid content of fish meal. This lower amino acid content causes the protein content of BSFL meal to be lower than fish meal. Fish meal is a superior protein source in omnivorous and carnivorous fish diets [27–29]. Therefore, the use of BSFL meal as a source of protein in fish and shrimp diets have a certain number of limitation in substituting the use of fish meal, but its depends on the species and environmental condition.

In this study, the growth performance and utilization of test diets containing BSFL meal by juvenile white shrimp were obtained as shown in Table 2. After the application of the test diets for 49 days, the final weight of white shrimp was obtained ranging from 9.2 - 10.1 g. The specific growth rate, weight gain and survival rate of white shrimp were not significantly different (P> 0.05) between treatments. Likewise, the utilization of test diets such as feed efficiency and protein efficiency ratio were not significantly different (P> 0.05) between treatments. This showed that the used of BSFL meal up to 22.5% in the feed formulation was still able to provide growth performance for white shrimp and feed utilization which were relatively the same as control diets (without BSFL meal) which used fish meal as its main protein source. The average specific growth rate of shrimp between treatments in this study ranged from 4.2-4.32 %/day, this value was almost the same as that reported by Cummins et al. [2] namely 3.82-4.07 %/day on the used of low-fat BSFL meal with a dosage of 0-21% BSFL meals in the feed formulation. Moreover, descriptively if we looket at the value of feed efficiency and protein efficiency ratio, we could see that it was tend to decrease at the level of 22.5% BSFL meals in the test diet. The ability of white shrimp to utilize feed containing BSFL meal up to 22.5% was probably related to the high protein content of the test diet (42.3%), with relatively high feed consumption (4.88%/day), and there was still a content of fish meal as much as 22.5% (substitute for the use of fish meal 50%) in diet, so that daily amino acid requirement could be fulfilled. White shrimp were able to utilize feed protein apart from fish meal sources [30–32]. Even in grow-out activities, white shrimp could utilize protein from vegetable materials such as soybean extract meal in high amount of, up to 56.46% of their diet [31]. White shrimp could also utilize protein concentrate from rice meal and it replaced the utilization of fish meal up to 50% using the rice meal concentrate protein as much as 22.85% in grow-out diet [33]. White shrimp could also use poultry by-product meal and replace the utilization of fish meal up to 80% by using poultry by-product meal as much as 31.37% in grow-out diet [3].

The BSFL meal used in this study contains 25.0% lipid (Table 1) with a fatty acid profile as in Table 3. The lipid content of BSFL meal was relatively high, and was one of the main sources of lipid, especially in the test diet containing 22.5% BSFL meal, namely about 5.6%. This also showed that the contribution of the lipid content of BSFL meal of around 5.6% or 62% of the total lipid in the TM22.5 test diet, and did not have a negative effect on the growth of testes animal, white shrimp juvenile. White shrimp can also make good use of diet that contains a mixture of soybean oil, beef tallow, and linseed oil compared to fish oil only [34]. White shrimp could also grow well when fed with vegetable-based ingredients with a source of 4.11% soybean oil and 0.46% fish oil [35] and is relatively the same as those that given fish oil (menhaden fish oil). While Lim et al. [36] reported that white shrimp required n-6 and n-3 essential fatty acids, n-3 was required for maximum growth, feed efficiency and survival rate. It was further reported that white shrimp may equally use n-3 and n-6 HUFA as a result of associated metabolic adaptations, specific environmental conditions or feeding habits of these species, and possible phylogenetic differences [37]. In general, marine shrimps have limited ability to synthesize n-3 PUFAs [38], and EPA and DHA were important for these animals including white shrimp [39]. Furthermore, it was reported that white shrimp seemed to be able to meet the needs of essential fatty acids when fed 0.5% n-3 HUFA feed [39].
Another important point from results of this study that the ability of white shrimp to utilize BSFL meal (the result of organic waste conversion) in practical diet will contribute evidently to reduce the use of fish meal which currently has a very high level of competition in its use. This will excite the community to process their organic waste into alternative protein sources for maintaining a high-value aquatic cultivation commodities, and create a positive impact on environmental sustainability.

Table 5. Growth performance of white shrimp fed test diets containing BSFL meals with different levels.

| Variables                      | TM0          | TM7.5        | TM15         | TM22.5       |
|--------------------------------|--------------|--------------|--------------|--------------|
| Initial body weight (g/ind)    | 1.22±0.04    | 1.20±0.02    | 1.22±0.13    | 1.18±0.10    |
| Final body weight (g/ind)      | 9.90±0.48    | 9.49±0.33    | 10.06±0.38   | 9.24±0.42    |
| Specific growth rate (%/day)   | 4.28±0.17    | 4.22±0.07    | 4.32±0.27    | 4.20±0.16    |
| Weight gain (%)                | 673.5±34     | 654.5±6.9    | 656.1±70.5   | 642.5±48.2   |
| Voluntary feed intake (%/day)  | 4.66±0.48    | 4.91±0.15    | 4.76±0.54    | 4.88±0.26    |
| Feed efficiency (%)            | 76.1±2.9     | 76.9±1.2     | 76.9±6.8     | 69.1±1.2     |
| Protein efficiency ratio       | 1.78±0.07    | 1.81±0.03    | 1.79±0.16    | 1.64±0.03    |
| Survival rate (%)              | 83.3±11.5 a  | 76.7±5.8 a   | 76.7±15.3 a  | 86.7±15.3 a  |

The values in the same row followed by the same superscript showed no significant difference (P>0.05).

The proximate whole body composition of white shrimp at the end of the study is presented in Table 6. The protein, lipid, crude fiber and ash content of the tested animals were not significantly different (P>0.05) between treatments. This showed that all the test diets that were applied did not have an effect that could change the proximate composition of the white shrimp’s whole body.

Table 6. Proximate composition (% dry matter) of the whole body of white shrimp fed test diets containing BSFL meal with different levels.

| Nutrient       | TM0          | TM7.5        | TM15         | TM22.5       |
|----------------|--------------|--------------|--------------|--------------|
| Protein        | 75.7±0.7 a   | 76.9±0.6 a   | 75.9±0.5 a   | 76.2±0.7 a   |
| Lipid          | 5.1±0.7 a    | 3.6±0.4 a    | 5.0±0.9 a    | 4.8±0.7 a    |
| Crude fibre    | 12.1±0.5 a   | 13.0±0.7 a   | 12.2±1.0 a   | 12.3±1.0 a   |
| Ash            | 11.5±0.7 a   | 11.5±1.1 a   | 12.1±1.5 a   | 11.1±0.6 a   |

The range value of water quality during the study was presented in Table 3. The value of the ammonia content was slightly starting to be high, namely 0.7254-1.7556 mg/L, especially when the nitrite content had reached 6.5076-7.1597 mg/L which occurred at the end of the study. This toxic nitrogen content could be one of the causes of shrimp mortality in addition to the cannibalism behavior when the shrimp was molting. Therefore, for the next activity, it was necessary to improve the cultivation media management system to maintain water quality conditions that were more optimal for the growth and survival of the tested animals.
Table 7. The value of the range of water quality variables during the study.

| Variables        | TM0     | TM7.5    | TM15    | TM22.5   |
|------------------|---------|----------|---------|----------|
| Salinity (ppt)   | 18-23   | 18-23    | 18-23   | 18-23    |
| Temperature (°C) | 27.5-30.5| 27.3-30.6| 27.3-30.4| 27.1-29.9|
| Dissolved oxygen (mg/L) | 4.48-5.94 | 4.88-6.22 | 4.77-6.47 | 4.63-6.24 |
| pH               | 7.50-8.10| 7.54-8.18| 7.50-8.19| 7.53-8.24 |
| NH3-N (mg/L)     | 0.0751-0.7254 | 0.1014-0.7535 | 0.0907-1.7556 | 0.0531-0.7078 |
| NO2-N (mg/L)     | 0.0017-6.5076 | 0.0731-6.3024 | 0.0458-6.4627 | 0.0693-7.1597 |
| NO3-N (mg/L)     | 0.0326-0.3132 | 0.0284-0.1046 | 0.0243-1.9158 | 0.1525-2.082 |
| Alkalinity (mg/L)| 186.76-235.48 | 182.70-243.60 | 190.82-227.36 | 190.82-231.42 |
| Total organic matter (mg/L) | 18.95-65.75 | 22.08-68.26 | 20.95-70.45 | 37.28-73.89 |
| Total Suspended solid (mg/L) | 39-43 | 48-133 | 38-57 | 53-69 |
| Phosphate (mg/L) | 0.0417-0.2445 | 0.0098-0.1270 | 0.0154-0.2928 | 0.1080-0.1503 |

4. Conclusion
Based on the results of this study, it was concluded that BSFM (fullfat) meal could be used up to level of 22.5% to partially replace (50%) the use of fish meal in practical diet for white shrimp grow-out.

Suggestion
It is necessary to test the use of BSFL meal in diet for grow-out white shrimp in pond.

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