JURNAL PERIKANAN
UNIVERSITAS GADJAH MADA
Terakreditasi Ristekdikti No: 158/E/KPT/2021
ISSN: 2502-5066 (Online)
ISSN: 0853-6384 (Print)
Vol. 26 (2), 193-201
DOI 10.22146/jfs.97636

Evaluation of BSF Larva Meal and Oil as Whiteleg Shrimp Feed on Growth Performance, Body Composition, and Health Response

Muhammad Rizal[1], Dewi Apri Astuti[1] & Melta Rini Fahmi[2]
[1]Department of Nutrition and Feed Technology, Faculty of Animal Scienc, IPB University, Bogor, West Java, Indonesia
[2]Research and Development Institution for Ornamental Fish, Ministry of Marine Affairs and Fisheries, Depok, West Java, Indonesia
*Corresponding author, email: mohriz19rizal@gmail.com
Submitted: 27 Juny 2024; Revised: 21 August 2024; Accepted: 18 September 2024; Published: 30 December 2024

ABSTRACT The goal of this study was to evaluate the use of BSF larvae meal and oil as feed components in order to determine their impact on *Litopenaeus vannamei* shrimp growth performance, nutritional composition, and health response. Three shrimp ponds, each with a diameter of 20 m, were stocked with 72.000 *Litopenaeus vannamei* post larvae (PL-8), resulting in a stocking density of 229 shrimp per square meter. The trial diets comprised three types: control diet (K), diet containing BSF larvae meal (A), and diet containing BSF larvae meal plus oil (B). The parameters observed in this study included shrimp growth and productivity, feed consumption, feed conversion ratio (FCR), survival rate, nutritional composition (proximate, amino acid profile and fatty acid profile), total bacteria in the shrimp's digestive tract, and total hemocyte count in the shrimp's hemolymph. It was found that subtituting fish meal with 34% BSF larvae meal did not affect the growth performance of shrimp raised in shrimp ponds, compared to the control treatment. The assessment of the amino acid profile has revealed minimal variation between treatment. Nevertheless, both treatments A and B exhibited a decreased concentration of unsaturated fatty acids, specifically omega-3 and omega-6, in comparison to control. Meanwhile, treatment B had the highest levels of saturated fatty acid and omega-9 fatty acid content.

Keywords: BSF larvaemeal; BSF larva oil; hemolymph; *Litopenaeus vannamei*; nutritional composition

INTRODUCTION

The whiteleg shrimp, scientifically known as *Litopenaeus vannamei*, has gained significant importance in worldwide aquaculture due to its notable attributes of rapid growth and exceptional tolerance to stressful conditions (FAO, 2020). According to the KKP (2020), Indonesia's shrimp production in 2020 reached 881.599 tons, with a total of 239.282 tons exported. This substantial level of output necessitates a considerable quantity of support in the form of shrimp feed.

Fish meals are the primary constituent in the fish feed business due to its suitable protein and amino acid content, as well as their great palatability. It is the main source of protein in shrimp feed formulations, typically making up 15-25% of the formulation (Xie *et al*., 2016)"ISSN":"00448486","abstract":"An 8-week feeding trial was conducted to evaluate the effect of fish-meal replacement on growth performance, whole body composition, apparent digestibility coefficients and anti-oxidative ability of juvenile Pacific white shrimp, Litopenaeus vannamei. Five practical diets were formulated to contain graded levels (5, 10, 15, 20 and 25%. The amount produced of fish meal and oil varies in response to fluctuations in the production of captured fish. The production of fish meal and oil reached its highest point in 1994, with a total of 30 million tonnes, but then decreased to fewer than 14 million tonnes by 2014 (FAO, 2020). There is a consistent decline in the production of fish meal and oil each year, while the demand for these resources, particularly for the animal feed and fish industries, is increasing due to their rapid growth. This phenomenon leads to a rise in the pricing of both products. To decrease the reliance of the shrimp feed industry on fish meal and oil, it is imperative to seek other sources of raw materials that can substitute or diminish the usage of fish meal without producing a substantial decline in shrimp performance. An available raw material alternative is meal made from the larvae of the black soldier fly (*Hermetia illucens*).

Research on the utilization of insect larvae as a protein source in animal and fish feed has been extensively conducted in the past ten years (Makkar *et al*., 2014)the feed being the most challenging because of the limited availability of natural resources, ongoing climatic changes and food-feed-fuel competition. The costs of conventional feed resources such as soymeal and fishmeal are very high and moreover their availability in the future will be limited. Insect rearing could be a part of the solutions. Although some studies have been conducted on evaluation of insects, insect larvae or insect meals as an ingredient in the diets of some animal species, this field is in infancy. Here we collate, synthesize and discuss the available information on five major insect species studied with respect to evaluation of their products as animal feed. The nutritional quality of black soldier fly larvae, the house fly maggots, mealworm, locusts-grasshoppers-crickets, and silkworm meal and their use as a replacement of soymeal and fishmeal in the diets of poultry, pigs, fish species and ruminants are discussed. The crude protein contents of these alternate resources are high: 42-63% and so are the lipid contents (up to 36% oil. The larvae of the black soldier fly (*Hermetia illucens*), sometimes known as BSF, have been effectively utilized as feed components for various animals, such as chickens (Schiavone *et al*., 2017), goats (Astuti & Wiryawan, 2022), pigs, and fish (Makkar *et al*., 2014)the feed being the most challenging because of the limited availability of natural resources, ongoing climatic changes and food-feed-fuel competition. The costs of conventional feed resources such as soymeal and fishmeal are very high and moreover their availability in the future will be limited. Insect rearing could be a part of the solutions. Although some studies have been conducted on evaluation of insects, insect larvae or insect meals as an ingredient

in the diets of some animal species, this field is in infancy. Here we collate, synthesize and discuss the available information on five major insect species studied with respect to evaluation of their products as animal feed. The nutritional quality of black soldier fly larvae, the house fly maggots, mealworm, locusts-grasshoppers-crickets, and silkworm meal and their use as a replacement of soymeal and fishmeal in the diets of poultry, pigs, fish species and ruminants are discussed. The crude protein contents of these alternate resources are high: 42-63% and so are the lipid contents (up to 36% oil. The study by Makkar *et al*. (2014) found that BSF larvae had a crude protein content of 42.1%, whereas defatted BSF larvae have a crude protein content of 56.9%. These protein levels are like soybean meals and somewhat lower than fish meals. The growth performance of Jian carp (*Cyprinus carpio var. Jian*) was not affected by substituting fish meal with LBSF defatted meal, even when the substitution was up to 100% (Li *et al*., 2017). According to a study by (Magalhães *et al*., 2017), it is possible to replace 19.5% of BSF larvae meal in feed or substitute 45% of fish meal in European seabass without any negative impact on growth performance. Furthermore, (Cummins *et al*., 2017) found that it is economically feasible to replace 10-17% of fish meal with black soldier fly larvae meal in vannamei shrimp.

In addition to their nutritional value, BSF larvae have been identified as having antimicrobial peptides (AMPs), which exhibit antibacterial properties and have the potential to be utilized as an alternative to antibiotics due to their low susceptibility for resistance development (Xia *et al*., 2021). Furthermore, it has been observed that BSF larvae consist of lauric acid in concentrations ranging from 3% to 52%, which is dependent upon the specific larval growth media utilized (Ewald *et al*., 2020). According to Matsue *et al*. (2019), lauric acid has been found to possess a wide range of antibacterial and antiviral properties.

Numerous variables can affect the performance of animals while consuming feed that contains black soldier fly larvae or prepupae. Initially, the higher level of fat in BSF might influence its ability to be digested and its overall taste. Furthermore, high ash level has the potential to decrease levels of feed consumption. Therefore, it is important to assess the adequacy of the amino acid composition in BSF for animals consuming feed that contains it, despite the fact that the crude protein level in BSF is substantially high (Barragan-Fonseca *et al*., 2017). Hence, before using BSF larvae meal as a feed raw material, it must be processed to reduce the fat content.

The utilization of BSF larval meal as a raw material for shrimp feed is expected to increase by incorporating defatted BSF larval meal and separately controlling the input of BSF larval oil as a feed ingredient. Research has documented the substitution of fish meal with BSF larval meal in whiteleg shrimp (*Litopenaeus vannamei*) feed (Chen *et al*., 2021; Cummins *et al*., 2017; Wang *et al*., 2021). However, its application in shrimp production (in ponds) has yet to be widely reported. This study was carried out on a large scale to investigate the application of BSF larvae meal and oil as feed components to evaluate the growth performance, nutritional value, and health response of whiteleg shrimp (*Litopenaeus vannamei*).

MATERIAL AND METHODS

Diet preparation and compositions

BSF larvae meal and oil in this experiment was provided localt by PT Bio Cycle Indo, Tapung District, Kampar Regency, Riau, Indonesia 28464. The diet used in this study consisted of pellets approximately 1.2-2 mm in size, which were produced at PT Satwa Boga Sempurna located in Cikupa, Tangerang, Banten, Indonesia 15710. These diets were formulated to fulfill the unique nutrient requirements of Whiteleg shrimp, Litopenaeus vannamei. Diet 1 (referred to as the control diet, K) was designed to be like a diet which is commercially available in Indonesia. Diet 2 (A) was formulated by substituting 34% of Fish meal with Black Soldier Fly (BSF) larvae meal at an inclusion level of 8.7%. Finally, diet 3 (B) was prepared on-site by incorporating 2% of BSF oil into Diet 2 (A). The method of blending the oil involves utilizing a mixer and incorporating BSF oil by spraying with a Pressure Sprayer. The composition of shrimp feed and its nutrition content is presented in (Table 1) and (Table 2). The experimental diets proximate are measured at BBPBAP laboratory located in Jepara, Indonesia. Meanwhile, the composition of amino acids and fatty acids was examined at Saraswanti Indo Genetech Laboratory in Bogor, Indonesia.

Feeding trial

The feeding trial was conducted at the Shrimp Farming Pond Unit, Brackish Water Aquaculture Fisheries Center (BPBAP) Situbondo, Gundil Village, Kendit, Situbondo Regency, East Java Indonesia. Three shrimp culture ponds, each with a diameter of 20 meters, were utilized. Each ponds were equipped with two pedal wheels and had both seawater and freshwater systems to maintan optimum salinity level. The water used for the trial originated from the nearby marine environment and undergoes treatment in a reservoir before being used. Each pond was stocked with 72,000 Litopenaeus vannamei post larvae (PL-8) from PT Windu Alam Sentosa (WAS) Rembang-Central Java-Indonesia. The stocking density was 229 shrimp per square meter.

A five-day period of acclimation to the control meal is done prior to the feeding trial. The test feed is introduced on the 40th day of growing in the pond, with the size of the feed adjusted according to the age/weight of the cultured shrimp. The test diets were administered at a rate of 5-3% of the shrimp's body weight per day, with the amount decreasing as the shrimp grew. The diets were given five times a day at specific times: 7:00, 10:00, 13:00, 16:00, and 19:00. This feeding schedule was followed for a duration of 40 days and recorded daily for each culture pond.

Water quality analysis and sample collection

Throughout the study, water quality parameters, including temperature, salinity, brightness, color, pH, and dissolved oxygen (DO) levels, are monitored twice daily. Water quality analysis, including measurements of nitrite levels, total ammonia nitrogen, organic matter levels, total bacteria, and total vibrios, is carried out on a weekly basis. Nitrite and total ammonia nitrogen are measured using spectrophotometer UV-VIS (Genesys 10S, Thermo Scientific, USA). Total bacteria and total vibrios are measured using total plate count (TPC) methode.

A total of 60 shrimp were measured for their weight and length in each trial pond to obtain the initial body weight data. Subsequently, periodic measurements were con-

Table 1. Composition of control feed (K); feed with BSF larva meal (A); and feed with meal and oil BSF larvae (B).

Raw Material	K		A		B	
	Vol (Kg)	Inclusion	Vol (Kg)	Inclusion	Vol (Kg)	Inclusion
Wheat flour	361	35.2%	333	32.8%	333	32.2%
Soybean meal, CP 46%	270	26.3%	298	29.4%	298	28.8%
Fish meal (CP 67)	250	24.4%	160	15.8%	160	15.5%
BSF meal	-	-	90	8.9%	90	8.7%
Squid liver meal	70	6.8%	70	6.9%	70	6.8%
Soy lecithin liquid	20	2.0%	20	2.0%	20	1.9%
Fish oil	10	1.0%	-	-	-	-
BSF oil	-	-	-	-	20	1.9%
Choline chloride 75%	1	0.1%	1	0.1%	1	0.1%
Water	25	2.4%	25	2.5%	25	2.4%
Mineral mix	2	0.2%	2	0.2%	2	0.2%
Calcium carbonate, $CaCO_3$	10	1.0%	10	1.0%	10	1.0%
Vitamin mix	2	0.2%	2	0.2%	2	0.2%
Aqua vit c	1	0.1%	1	0.1%	1	0.1%
L-threonine (promois)	0.5	0.0%	0,9	0.1%	0.9	0.1%
Toxin binder	1	0.1%	1	0.1%	1	0.1%
Pellet binder (PMC)	1	0.1%	1	0.1%	1	0.1%
Total	1.025	100%	1.015	100%	1.035	100%

Table 2. Feed proximate and amino acid compositions (dry weight).

Nutrients	Treatment		
	K	A	B
Proximate			
Water Content (WC) %	10.18	10.79	10.72
Ash %	10.75	11.41	11.18
Crude Fat %	9.03	11.07	12.52
Crude Protein %	38.08	37.31	36.84
Crude Fibre %	2.10	2.41	2.28
Amino Acid			
L-Serine (%)	1.93	1.72	1.62
L-Glutamic Acid (%)	7.00	6.33	6.25
L-Phenylalanine (%)	1.35	1.21	1.15
L-Isoleucine (%)	1.37	1.27	1.25
L-Valine (%)	1.61	1.57	1.53
L-Alanine (%)	1.95	1.78	1.76
L-Arginine (%)	1.84	1.57	1.52
Glycine (%)	1.84	1.64	1.60
L-Lysine (%)	2.94	2.47	2.43
L-Aspartic Acid (%)	3.84	3.35	3.33
L-Leucine (%)	2.76	2.45	2.39
L-Tyrosine (%)	0.81	0.78	0.74
L-Proline (%)	1.85	1.74	1.68
L-Threonine (%)	1.69	1.50	1.43
L-Histidine (%)	0.72	0.65	0.62

ducted every 10 days on a sample of 60 shrimps in each trial pond. This involved randomly selecting 20 shrimps at three different locations inside each pond. At the end of the trial period (before harvesting), health and immunity of the shrimp were assessed by measuring the total gastrointestinal bacteria and total hemocyte count (THC) at BPAP Situbondo Laboratory, West Java, Indonesia. Five shrimps were taken from three randomly selected sites in each pond and sent to the laboratory for further analysis.

At the end of the study, the shrimp were harvested. Subsequently, the shrimp were weighed, and a random sample of roughly 1 kg from each pond was collected. These samples were then placed in plastic containers and frozen for future preparation. Subsequently, a quantity of 300-400 grams of shrimp was processed by blending and homogenizing it with a homogenizer (Wiggen Hauser, Germany). The resulting mixture was then freeze dried using a laboratory freeze drier (Martin Christ, Germany).

Laboratory testing

For THC testing, hemolymph was collected from three randomly selected shrimp per dietary treatment at the conclusion of the growth trial using a syringe and a 25-gauge needle. A 1 mL syringe was filled in advance with 0.5 mL of an anti-coagulant and then used to extract around 0.5 mL of hemolymph from each shrimp. The anticoagulant solution consists of 30 mM Sodium Citrate Tribasic Dihydrate (Sigma S4641), 0.34 M Sodium chloride (NaCl), and 10 mM EDTA – Ethylene Diamine Tetraacetic Acid (Sigma, E9884) diluted in distilled water. The hemolymph, along with an anti-coagulant solution, was diluted in 150 µL of formaldehyde (4%). Subsequently, 20 µL of the resulting solution were applied to a hemocytometer (Neubauer) for the purpose of determining the total hemocyte count (THC). The THC was measured using an optical microscope (Olympus, BX53) at a magnification of 400x.

To measure the total amount of bacteria in the gastrointestinal tract, a validated method, total plate count (TPC), was performed in the laboratory. The sample was diluted many times using sterile buffer. A volume of 0.1 mL of the sample is transferred onto the agar surface that has been prepared on a petri dish, and then it is evenly distributed using a sterile glass rod. After the samples are allowed to dry, they are transferred to an incubator set at an appropriate temperature for growth. Following the incubation time, the number of colonies is quantified to determine the quantity of microorganisms contained in the initial sample.

Validated procedures are utilized for proximal analysis. The moisture level was assessed using the thermogravimetry method after drying the samples at 105 °C in an oven (Memmert, Germany) until a constant weight was achieved. The crude protein content was determined using the DUMAS procedure with the DUMATERM instrument (Gerhardt, Germany). Crude lipids were quantified by extracting the sample with n-hexane using the Soxtherm apparatus (Gerhardt, Germany). Crude fiber content was determined by subjecting the sample to alternating acid and base washing using the FIBRETHERM instrument (Gerhardt, Germany). The ash content was determined by combusting the sample in a furnace (Barnstead Thermolyne, CA, USA) at 550 °C for 8 hours. The analysis of amino acid and fatty acid profile was conducted at the Saraswanti Indo Genetech Laboratory in Bogor, Indonesia.

Statistical analysis

Growth and shrimp pond prodution parameters, whole body proximate, THC, total gastrointestinal bacteria were analyzed using a one-way analysis of variance (ANOVA) to determine significant differences among treatments followed by Duncan's HSD tes to determine the difference between treatment means among the treatments with ignificance level of P>0,05. All statistical analysis was carried out using the SAS on Demand for Academics. Data on water quality of rearing media, feed amino acid profile, shrimp amino acid profile, and shrimp fatty acid profile were analyzed descriptively.

RESULTS AND DISCUSSION

Pond water quality

Shrimp production was carried out in open ponds, therefore making it highly susceptible to external influences, particularly the water quality of the pond. Regular monitoring of water quality is conducted, and the results are displayed in Table 3. Most water quality indicators for shrimp rearing fall within the recommended range (Ferreira *et al*., 2011), including temperature between 18–33 °C, dissolved oxygen levels at 2.5–10 mg/L, pH levels between 7–10, and total alkalinity between 50–150 mg/L CaCO3. The levels of bacteria and vibrio exceed the recommended range, which is less than 104 cfu/mL and less than 103 cfu/mL respectively (Carbajal-Hernández *et al*., 2013). The utilization of probiotics in shrimp rearing, aimed at facilitating the breakdown of feed waste and fecal matter, is believed to be the primary cause for the observed increase in both total bacteria and total vibrio levels. The levels of bacteria and vibrio in the pond with diet B were observed lower compared to both the control (K) pond's water and the pond with diet A. BSF oil is rich in lauric acid, which possesses antimicrobial characteristics (Matsue *et al*., 2019). It is possible that some of the BSF oil could be transferred from the feed to the water, resulting in a decrease in the overall levels of bacteria and vibrio in the pond.

Growth and production performance

Although numerous studies have demonstrated the potential of BSF as an ingredient in shrimp *Litopenaeus vannamei*, the majority of the research conducted to date has been in laboratory settings using small culture tanks under controlled conditions (Chen *et al*., 2021; Cummins *et al*., 2017; Wang *et al*., 2021).Employing the usage of an outdoor pond as the cultivation system might result in diverse observations regarding the growth and health condition of shrimp.

The data from (Table 4) on shrimp production parameters indicate that, after a trial period of 40 days, the final body weight of shrimp fed with the control diet was higher than that of shrimp fed with diet A or diet B. However, shrimp fed with a diet containing black soldier fly larvae (BSFL) meal and BSFL oil (diet B) exhibited a higher survival rate (SR = 95%) compared to the control diet K (SR = 77%) and diet A (SR = 75%). Additionally, the pond using diet B had the lowest feed conversion ratio (FCR) value of 1.49. The high survival rate is believed to be due to the low mortality rate of shrimp, whether caused by cannibal-

Table 3. Water quality of rearing pond.

Water Quality Parameters	Treatment		
	K	A	B
pH			
Morning	7.6-8.2	7.7-8.3	7.7-8.2
Afternoon	7.5-8.7	7.7-8.7	7.7-8.6
Salinity (ppt)			
Morning	23-30	16-30	25-30
Afternoon	25-31	25-30	24-30
Dissolved Oxygen (mg/L)			
Afternoon	4.39-7.14	4.07-6.78	4.1-6.71
Night	3.47-5.63	3.75-6.28	3.09-5.58
Temperature (°C)			
Afternoon	27.7-31.8	27.8-31.7	27.8-31.9
Night	27.3-30.9	27.0-31.0	27.5-31.1
NO_2^- (mg/L)	1.14-13.96	2.29-12.1	0.522-2.03
Total Ammonia Nitrogen (mg/L)	0.084-2.75	0.064-3.4	0.082-3.2
Alkalinity (mg/L $CaCO_3$)	124-188	128-194	132-168
Organic Matter (mg/L)	99-115	90-111	94.8-121
Total bacteria (x 10^4 cfu/mL)	2.00-8.00	2.00-4.00	0.40-4.80
Total vibrio (x 10^3 cfu/mL)	1.2-8.00	0.24-1.60	0.4-2.00

Control diet (K); diet with BSF larvae meal (A), and diet with BSF larvae meal and oil (B).

ism or other factors. However, further testing is required to determine if BSFL oil influences the level of shrimp cannibalism.

The results of the ANOVA test, followed by the Duncan test, indicated that there was no statistically significant difference ($p<0.05$) in weight growth between the shrimp fed with control diet K and the shrimp fed with diet A at the conclusion of the trial period. Utilizing black soldier fly larvae meal at inclusion level of 8.9% or substituting 34% of fish meal did not result in a decrease in shrimp growth performance. Study carried out by (Novriadi *et al*., 2024) reported the optimal inclusion range of BSFM from 0.5% to 5% as well as the combination of BSFM (0.5%) and graded levels of BSFO from 0.5% to 5% could improve the growth performance and health. Meanwhile, (Chen *et al*., 2021) found that shrimp growth was not adversely affected by the substitution of 20% fish meal with BSF meal. In another experiment, (Wang *et al*., 2021) found that it is possible to substitute up to 60% of fish meal with defated BSF larave meal without observing any negative impact on growth performance.

The weight gains of shrimp at the en of trial utilizing diet B showed significantly lower results ($p<0.05$) compared to the weight gains of shrimp fed with control diet K and diet A (Table 5). Nevertheless, an increased overall biomass is evident when examining the pond production data

Table 4. Shrimp production data using control diet (K); diet with BSF larvae meal (A); and diet with BSF larvae meal and oil (B).

Shrimp Production Parameters	Treatment		
	K	A	B
Estimated initial population	72.000	72.000	72.000
Initial average body weight (g)	4.76	4.46	4.41
Final average body weight (g)	14.205	13.677	13.075
Estimate final population	55.607	52.990	68.645
Total biomass (Kg)	752	689	866
Total test biomass (Kg)	488	452	564
Total feed intake (Kg)	836	856	843
FCR	1.71	1.89	1.49
SR (%)	77%	75%	95%

Control diet (K); diet with BSF larvae meal (A), and diet with BSF larvae meal and oil (B).

related to diet B. Furthermore, the survival rate (SR) of shrimp in ponds fed with diet B was 95%, which is greater than the survival rates of shrimp fed with control diet K and diet A, which were 77% and 75% respectively. In addition, the FCR value of diet B treatment was 1,49, which was 0,22 lower than the FCR value of the control diet treatment. Therefore, it can be inferred that the restricted growth in shrimp trials using feed B was due to the larger shrimp population in pond B compared to ponds K and A. The shrimp length did not exhibit a statistically significant difference ($p<0.05$) among the three diet treatments.

Shrimp nutrition profile test results

Data on the nutritional content of shrimp from testing results, including proximate and amino acid test results, are presented in (Table 6). Duncan test results show that there is a significant difference ($p<0.05$) in the crude protein test results, with the crude protein content of shrimp fed with diet A having the highest content of 76,4% and the crude protein content of shrimp fed with diet B was the lowest with a value of 74,49%. The crude fiber content in feed treatment K was 6.79%, which was also significantly different ($p<0.05$) compared to feed treatment A and feed B. As for the ash and crude fat content, there were no significant differences ($p<0.05$). The study's results (Usman *et al*., 2021) reported no difference in the protein, fat, crude fiber, and ash content of shrimp-fed feed containing BSF larval meal, even up to 22.5% consumption. The amino acid profile of shrimp did not appear to differ much between treatments.

Table 5. Shrimp weight and length growth performance (means ± s.d.).

	Treatment			P-Value
	K	A	B	
Weight (g)				
H10	3.17±1.46	2.94±1.38	3.04±1.03	0,628
H20	6.72^{a}±1.64	6.04^{b}±1.32	6.19^{b}±1.06	0,019
H30	8.47^{a}±1.43	7.73^{b}±1.17	8.01^{ab}±1.28	0,008
H40	9.44^{a}±1.47	9.22^{a}±1.60	8.67^{b}±1.73	0,024
Lenght (mm)				
H10	17.3±6.77	18.2±6.50	16.1±5.34	0,190
H20	31.6±5.55	31.9±5.50	29.9±4.02	0,085
H30	39.6±4.75	39.1±4.05	39.1±4.06	0,745
H40	41.9±4.47	42.0±4.97	40.6±5.13	0,199

Control diet (K); Diet with BSF larvae meal (A); and diet with BSF larvae meal and oil (B); H10-40: data collected at 10, 20, 30, 40 days after test feed aplication; The values in the same row followed by the same superscript showed no significant difference ($P> 0.05$).

The analysis of the shrimp fatty acid profile (Table 7) reveals that the levels of unsaturated fatty acids in shrimp fed to diet A and diet B reduced by 0,24% and 0,51% accordingly. In addition, the levels of omega-3 and omega-6 fatty acids in shrimp fed with both diet A and diet B similarly reduced. In contrast, shrimp fed with diet B exhibited a saturated fatty acid level of 1.44%, indicating a 0.08% increase compared to the control diet K treatment. The levels of lauric acid and omega-9 in shrimp which were fed with diet B also showed an increase when compared to the control diet K treatment. It is believed that this originates from the oil of BSF larvae, which has substantially elevated quantities of omega-9 and lauric acid. These findings indicate that the utilization of BSF larval oil leads to alterations in the fatty acid composition of the cultured shrimp produced.

Shrimp health status

The health evaluation of the shrimp involved measuring the total bacterial presence in the shrimp's digestive tract and the total hemocyte count (THC) in the shrimp's hemolymph. Based on the data obtained in (Table 8), there was no significant difference ($p<0.05$) in the total bacteria and total vibrios found in the shrimp digestive system across all treatments.The presence of microbiota in the digestive tract is a contributing component to the overall well-being of shrimp (host) (Hou *et al*., 2020).

Hemocytes, found in crustaceans and other invertebrates, are crucial components of the animal's immune system. They are involved in several immune functions such as phagocytosis, encapsulation, and destruction of foreign cells (Johansson *et al*., 2000). Therefore, the

Table 6. Composition of proximate and amino acids (dry weight) of the whole body of shrimp with test diet containing BSF larvae meal and oil.

Nutrients	Treatment			P-Value
	K	A	B	
Ash %	14.55±0.31	13.84±0.22	14.71±0.62	0.093
Total Fat %	4.17	3.80	3.74	
Crude Protein %	75.47^{b}±0.43	76.40^{a}±0.16	74.49^{c}±0.42	0.002
Crude Fibre%	6.79^{a}±0.12	5.72^{b}±0.55	5.93^{b}±0.15	0.017

Amino acid			
L-Serine (%)	2.68	2.75	2.80
L-Glutamic Acid (%)	9.39	8.99	9.15
L-Phenylalanine (%)	2.14	2.28	2.30
L-Isoleucine (%)	2.13	2.12	2.11
L-Valine (%)	2.57	2.56	2.55
L-Alanine (%)	3.89	3.83	3.73
L-Arginine (%)	4.30	4.58	4.64
Glycine (%)	5.14	5.35	5.65
L-Lysine (%)	5.61	5.31	5.18
L-Aspartic Acid (%)	6.07	5.90	5.89
L-Leucine (%)	4.30	4.28	4.30
L-Tyrosine (%)	1.61	1.71	1.72
L-Proline (%)	3.39	3.26	3.25
L-Threonine (%)	2.68	2.78	2.79
L-Histidine (%)	1.10	1.19	1.19

Control feed (K); feed with BSF larvae meal (A); and feed with BSF larvae meal and oil (B); The values in the same row followed by the same superscript showed no significant difference (P> 0.05).

quantity of hemocytes can serve as an indicator of the health status of shrimp (Sritunyalucksana *et al*., 2005). The study found that the THC value in shrimp fed with the control diet K was significantly lower ($p<0.05$) compared to shrimp fed with both diet A and diet B. A higher THC score signifies an improved immune system capacity under similar conditions.

Table 7. Fatty acid composition (dry weight) of whole body of shrimp with test feed containing BSF larvae meal.

Fatty Acids	Treatment		
	K	A	B
C 18:2 W6C (C-Linoleic Acid) (%)	0.99	0.79	0.80
C 18:1 W9C (C-Oleic Acid) (%)	0.88	0.70	1.09
C 20:5 W3 (Eicosatpentaenoic Acid) (%)	0.53	0.52	0.21
C 16:1 (Palmitoleic Acid) (%)	0.06	0.03	nd
C 20:4 W6 (Arachidonic Acid) (%)	nd	0.09	nd
Omega 6 Fatty Acids (%)	0.99	0.88	0.80
C 20:2 (Eicosadienoic Acid) (%)	nd	0.07	nd
DHA (%)	0.27	0.28	0.13
Omega 3 Fatty Acids (%)	0.88	0.85	0.41
C 11:0 (Undecanoic Acid) (%)	nd	0.01	nd
C 18:3 W3 (Linolenic Acid / W3) (%)	0.08	0.05	0.07
Polyunsaturated Fat (%)	1.88	1.81	1.21
C 22:6 W3 (Docosahexanoic Acid) (%)	0.27	0.28	0.13
C 18:0 (Stearic Acid) (%)	0.31	0.34	0.23
C 16:0 (Palmitic Acid) (%)	0.96	0.83	1.07
Unsaturated Fat (%)	2.81	2.57	2.30
Omega 9 Fatty Acids (%)	0.88	0.70	1.09
C 15:0 (Pentadecanoic Acid) (%)	nd	0.01	nd
Arachidonic acid (%)	nd	0.09	nd
C 14:0 (Myristic Acid) (%)	0.05	0.04	0.07
EPA (%)	0.53	0.52	0.21
C 12:0 (Lauraic Acid) (%)	0.03	0.01	0.07
Monounsaturated Fat (%)	0.93	0.76	1.09
Saturated Fat (%)	1.36	1.23	1.44

Control diet (K); diet with BSF larvae meal (A); and diet with BSF larvae meal and oil (B); nd = not detected.

Tabel 8. Total bacteria and total gastrointestinal vibrio as well as total hemocyt count of shrimp hemolymp with test feed containing BSF larvae meal, (mean ± s.d.)

Parameters	Treatment			P-Value
	K	A	B	
Total bacteria (10^8 CFU/g)	1.43±0.49	2.00±0.26	1.87±0.64	0.369
Total vibrio (10^7 CFU/g)	1.47±0.35	1.77±0.15	1.90±0.50	0.389
THC (10^6 cell/mL)	9.30^b±0.42	20.53^a±3.96	21.17^a±2.87	0.016
- Granule (10^6 Cell/mL)	5.25±0.21	12.0±5.46	14.03±0.38	0.090
- Hyalin (10^6 Cell/mL)	4.05±0.21	8.53±2.20	7.13±2.93	0.199

Control diet (K); diet with BSF larvae meal (A); and diet with BSF larvae meal and oil (B); The values in the same row followed by the same superscript showed no significant difference (P> 0.05).

CONCLUSION AND RECOMMENDATION

Conclusion

Whiteleg shrimp (*Litopenaeus vannamei*) are raised in production ponds or shrimp ponds, replacing 34% fish meal with 8.9% BSF larval food enhances the crude protein content and decreases the crude fiber content. This substitution does not have any adverse effects on the growth performance of the shrimp. Utilizing 2% BSF larval oil in the diet can induce changes in the fatty acid composition of cultured shrimp.

Recommendation

The total bacterial count in the experimental pond treated with BSF larvae meal and oil exhibited a tendency to be lower compared to the control treatment. Additional research is deemed necessary to investigate this effect. This can be done by either applying BSF flour and oil in as feed or by directly applying them in the water used for aquacultures.

AUTHORS' CONTRIBUTIONS

Each author's contribution to the manuscript's analysis technique, English grammar check, and proofreading.

ACKNOWLEDGMENT

We would like to thank Brackish Water Aquaculture Fisheries Center (BPBAP) Situbondo for providing shrimp rearing and fish healt laboratory facilities in this study. We also like to thank Main Center of Brackish Water Aquaculture Fisheries (BBPBAP) Jepara for providing facilities dor proximate analysis and sample preparation for ammino acid and fatty acid analysis. Some part of this study was funded by Matching Fund 15222/IT3.L2/HK.07.00/P/B/2021.

REFERENCES

Astuti, D.A & K.G. Wiryawan. 2022. Black soldier fly as feed ingredient for ruminants. Animal Bioscience. 35 (2): 356-363. https://doi.org/10.5713/AB.21.0460

Barragan-Fonseca, K.B., M. Dicke & J.J.A. van Loon. 2017. Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed - A review. Journal of Insects as Food and Feed. 3 (2): 105-120. https://doi.org/10.3920/JIFF2016.0055

Carbajal-Hernández, J.J., L.P. Sánchez-Fernández, L.A. Villa-Vargas, J.A. Carrasco-Ochoa & J.F. Martínez-Trinidad. 2013. Water quality assessment in shrimp culture using an analytical hierarchical process. Ecological Indicators. 29: 148-158. https://doi.org/10.1016/j.ecolind.2012.12.017

Chen, Y., S. Chi, S. Zhang, X. Dong, Q. Yang, H. Liu, B. Tan & S. Xie. 2021. Evaluation of the dietary black soldier fly larvae meal (*Hermetia illucens*) on growth performance, intestinal health, and disease resistance to *Vibrio parahaemolyticus* of the whiteleg shrimp (*Litopenaeus vannamei*). Frontiers in Marine Science. 8. https://doi.org/10.3389/fmars.2021.706463

Cummins, V.C., S.D. Rawles, K.R. Thompson, A. Velasquez, Y. Kobayashi, J. Hager & C.D. Webster. 2017. Evaluation of black soldier fly (*Hermetia illucens*) larvae meal as partial or total replacement of marine fish meal in practical diets for Whiteleg shrimp (*Litopenaeus vannamei*). Aquaculture. 473: 337-344. https://doi.org/10.1016/j.aquaculture.2017.02.022

Ewald, N., A. Vidakovic, M. Langeland, A. Kiessling, S. Samples & C. Lalander. 2020. Fatty acid composition of black soldier fly larvae (*Hermetia illucens*) – Possibilities and limitations for modification through diet. Waste Management. 102: 40-47. https://doi.org/10.1016/j.wasman.2019.10.014

FAO. 2020. The State of World Fisheries and Aquaculture 2020. In The State of World Fisheries and Aquaculture 2020. FAO. https://doi.org/10.4060/ca9229en

Hou, D., R. Zhou, S. Zeng, D. Wei, X. Deng, C. Xing, L. Yu, Z. Deng, H. Wang, S. Weng, J. He & Z. Huang. 2020. Intestine bacterial community composition of shrimp varies under low- and high-salinity culture conditions. Frontiers in Microbiology. 11: 1-10. https://doi.org/10.3389/fmicb.2020.589164

Johansson, M.W., P. Keyser, K. Sritunyalucksana & K. Söderhäll. 2000. Crustacean haemocytes and haematopoiesis. Aquaculture. 191 (1-3): 45-52. https://doi.org/10.1016/S0044-8486(00)00418-X

Li, S., H. Ji, B. Zhang, J. Zhou & H. Yu. 2017. Defatted black soldier fly (*Hermetia illucens*) larvae meal in diets for juvenile Jian carp (*Cyprinus carpio* var. Jian): Growth performance, antioxidant enzyme activities, digestive enzyme activities, intestine and hepatopancreas histological structure. Aquaculture. 477: 62-70. https://doi.org/10.1016/j.aquaculture.2017.04.015

Magalhães, R., A. Sánchez-López, R.S. Leal, S. Martínez-Llorens, A. Oliva-Teles & H. Peres. 2017. Black soldier fly (*Hermetia illucens*) pre-pupae meal as a fish meal replacement in diets for European seabass (*Dicentrarchus labrax*). Aquaculture. 476: 79-85. https://doi.org/10.1016/j.aquaculture.2017.04.021

Makkar, H.P.S., G. Tran, V. Heuzé & P. Ankers. 2014. State-of-the-art on use of insects as animal feed. In Animal Feed Science and Technology. 197: 1-33). Elsevier B.V. https://doi.org/10.1016/j.anifeedsci.2014.07.008

Matsue, M., Y. Mori, S. Nagase, Y. Sugiyama, R. Hirano, K. Ogai, K. Ogura, S. Kurihara & S. Okamoto. 2019. Measuring the antimicrobial activity of lauric acid against various bacteria in human gut microbiota using a new method. Cell Transplantation. 28 (12): 1528-1541. https://doi.org/10.1177/0963689719881366

Novriadi, R., S. Davies, K.I.K. Triatmaja, M. Hermawan, E.K.M. Kontara, B. Tanaka, A. Rinaldy & J.E. Nugroho. 2024. Black soldier fly (*Hermetia illucens*) as an alternative to marine ingredients elicits superior growth performance and resistance to *Vibrio harveyi* infection for Whiteleg shrimp (*Litopenaeus vannamei*). Turkish Journal of Fisheries and Aquatic Sciences. 24 (1). https://doi.

org/10.4194/TRJFAS24343

Schiavone, A., M. De Marco, S. Martínez, S. Dabbou, M. Renna, J. Madrid, F. Hernandez, L. Rotolo, P. Costa, F. Gai & L. Gasco. 2017. Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: Apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. Journal of Animal Science and Biotechnology. 8 (1). https://doi.org/10.1186/s40104-017-0181-5

Sritunyalucksana, K., W. Gangnonngiw, S. Archakunakorn, D. Fegan & T.W. Flegel. 2005. Bacterial clearance rate and a new differential hemocyte staining method to assess immunostimulant activity in shrimp. Diseases of Aquatic Organisms. 63 (1): 89-94. https://doi.org/10.3354/dao063089

Usman, U., M. Fahrur, K. Kamaruddin, A.I.J. Asaad & M.R. Fahmi. 2021. The utilization of black soldier fly larvae meal as a substitution of fish meal in diet for white shrimp, *Litopenaeus vannamei*, grow-out. IOP Conference Series: Earth and Environmental Science. 860 (1): 1-10. https://iopscience.iop.org/article/10.1088/1755-1315/860/1/012023

Wang, G., K. Peng, J. Hu, W. Mo, Z. Wei & Y. Huang. 2021. Evaluation of defatted *Hermetia illucens* larvae meal for *Litopenaeus vannamei*: Effects on growth performance, nutrition retention, antioxidant and immune response, digestive enzyme activity and hepatic morphology. Aquaculture Nutrition. 27 (4): 986-997. https://doi.org/10.1111/anu.13240

Xia, J., C. Ge & H. Yao. 2021. Antimicrobial peptides from black soldier fly (*Hermetia illucens*) as potential antimicrobial factors representing an alternative to antibiotics in livestock farming. Animals. 11 (7). https://doi.org/10.3390/ani11071937

Xie, S-W., Y-J. Liu, S. Zeng, J. Niu & L-X. Tian. 2016. Partial replacement of fishmeal by soy protein concentrate and soybean meal-based protein blend for juvenile Whiteleg shrimp, *Litopenaeus vannamei*. Aquaculture. 464: 296-302. https://doi.org/10.1016/j.aquaculture.2016.07.002

How to cite this article Rizal, M., D.A. Astuti & M.R. Fahmi. 2024. Evaluation of BSF larva meal and oil as white shrimp feed on growth performance, body composition, and health response. Jurnal Perikanan Universitas Gadjah Mada. 26 (2): 193-201. https://doi.org/10.22146/jfs.97636