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RESEARCH PAPER

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Black Soldier Fly (*Hermetia illucens*) as an Alternative to Marine Ingredients Elicits Superior Growth Performance and Resistance to *Vibrio harveyi* Infection for Pacific White Shrimp (*Litopenaeus vannamei*)

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Abstract

To assess the feasibility of incorporating insect meal and oil into diets for Pacific white shrimp *Litopenaeus Vannamei*, a trial was conducted to measure growth and feed performance metrics within balanced iso-nitrogenous and iso-lipid (36% protein and 6% lipid) experimental diets formulated with standard commercially sources ingredients. Incremental levels of BSF larval meal (BSFM, 0.5 to 5%) were used to partially substitute the use of fishmeal (FM) in the diet. In addition, next group of dietS were designed by using incremental levels of BSF larval oil (BSFO (0.5 to 5%) with 0.5% BSFM as a reference to partially and completely replace the use of fish oil (FO) within the diet. Ten dietary treatments were evaluated as four replicates in 40 Hapa ponds assigned randomly. Shrimp of initial mean weight of 0.97 g were fed by hand over 90 days with feed intake based on an FCR of 1.5 and all parameters recorded to determine response. Inclusion of up to 5% of both BSF meal and oil improved performance with respect to growth rate and feed utilization efficiency FCR. Total hemocyte counts and lysozyme activity reflected these trends displaying advantages of BSF diet groups compared to the basal fed group of *L. Vannamei.* After the growth trial, a disease challenge test was undertaken using an infection model with *Vibrio harveyi* at a concentration of 10^5 CFU shrimp^{-1} under controlled laboratory settings. As such, several indices of health status were recorded that included hemocyte counts, lysozyme activity as well as histopathology of the hepatopancreas that is a prime indicator of the progression of disease and a reflection on health status. Insect meal and oil inclusions increased survival from 40% to 60 - 80% and in accordance, raised the immune response and improved histopathological profiles of hepatopancreas tissues.

Introduction

The global shrimp farming industry has grown exponentially in recent decades, with a significant focus on the rearing of *Litopenaeus vannamei*, commonly known as Pacific white shrimp or white leg shrimp. *L. vannamei* has become a popular species for farming due to its fast growth rate, high yield, and adaptability to different environmental conditions (Amelia, Yustiati, Andriani, 2021; Karthik, Pushpam, Chelvan, Vanitha, 2015; Liao, Chien, 2011). The scale of the global shrimp farming industry has witnessed remarkable expansion. According to the FAO (2022), global shrimp production reached approximately 4.4 million metric tons in 2020, with a value of over USD 40 billion, making it one of the most valuable fisheries commodities globally. Countries such as Ecuador, China, Vietnam, India, and Indonesia are major producers of farmed shrimp (Davis, Boyd, Godumala, Mohan, Gonzalez, Duy, Ahyani, Shatova, Wakefield, Harris, 2022; Geetha, Ravisankar, Patil, Avunje, Vinoth, Sairam, Vijayan, 2020; Kakoolaki, Ebne al-Torab, Ghajari, Anvar, Sepahdari, Ahari, Hoseinzadeh, 2020).

The major ingredients used in shrimp feeds in the industry typically include fishmeal (FM), fish oil (FO), and plant-based protein sources such as soybean meal

(SBM), corn gluten meal (CGM), and wheat flour (WF) (NRC, 2011). FM and FO, derived from wild-caught pelagic fish species, have been traditionally used as key protein and lipid sources in shrimp feeds due to their excellent source of indispensable amino acids, essential fatty acid, vitamins, minerals, and also enhance the attractability as well as the palatability of the diet (Davis, Arnold, 2000; Rice, 2009). However, the increasing demand for these ingredients has led to concerns about overfishing and the sustainability of these resources (Tacon, Metian, 2008; Tacon, Metian, McNevin, 2022). Additionally, the use of FM and FO in shrimp feeds has been associated with issues such as high feed costs, potential contamination with harmful substances, and environmental impacts such as habitat destruction and overfishing of marine resources (Al Eissa, Chen, Brown, Huang, 2022; Chen, Chi, Zhang, Dong, Yang, Liu, Zhang, Deng, Tan, Xie, 2021).

To address these sustainability concerns, several alternatives have been proposed for shrimp feed ingredients. One notable approach is the use of alternative protein sources such as plant-derived proteins, microbial-based proteins, and insect-based proteins. Plant-based proteins such as SBM, CGM, and advanced products of such vegetable-protein sources have been widely used in shrimp feeds and have been effective in supporting shrimp growth and health (Manikandan, Felix, 2020; Novriadi, Herawati, Prayitno, Windarto, Mertz, Nguyen Duy, 2022; Novriadi, Istiqomah, Isnansetyo, Balk, Jolly-Breithaupt, Davies, 2023; Shao, Wang, Liu, Jiang, Wang, Wang, 2019; Yao, Zhang, Li, He, Wang, Leng, 2020). Microbial-based proteins such as single-cell proteins derived from yeast or bacteria have also shown promise as sustainable alternatives to fishmeal in shrimp feeds (Eroldoğan, Glencross, Novoveska, Gaudêncio, Rinkevich, Varese, de Fátima Carvalho, Tasdemir, Safarik, Nielsen, 2022). Additionally, insect-based proteins, such as black soldier fly (BSF, *Hermetia illucens*) meal, have been explored as a potential alternative to fishmeal in shrimp feeds, as they are highly nutritious and can be produced using organic waste as a feedstock (Chen, Chi, Zhang, Dong, Yang, Liu, Tan, Xie, 2021; Mousavi, Zahedinezhad, Loh, 2020; Surendra, Tomberlin, van Huis, Cammack, Heckmann, Khanal, 2020). Insect protein typically BSF larval meal has also been extensively investigated as a viable ingredient for many fish species (Alfiko, Xie, Astuti, Wong, Wang, 2022; Cho, Bae, Hwang, 2022).

The aquaculture sector can benefit from incorporating insect protein and oil into shrimp feed, as supported by scientific literatures (Chen, Chi, Zhang, Dong, Yang, Liu, Tan, Xie, 2021; Mohan, Rajan, Muralisankar, Ganesan, Sathishkumar, Revathi, 2022; Richardson, Dantas-Lima, Lefranc, Walraven, 2021). Firstly, in terms of production sustainability, BSF were able to convert the large quantities of organics and substrates in by-product streams from food processing, including palm kernel meal (PKM), to edible proteins as a high-quality source of essential amino acids (Li, Ji, Zhang, Tian, Zhou, Yu, 2016; Zheng, Li, Zhang, Yu, 2012). Research has shown that BSF larval meal can be a suitable replacement for fish meal, a commonly used protein source in shrimp feed, due to its high protein content, amino acid profile, and digestibility (He, Liu, Zhang, Wang, Wang, Zuo, Jiang, 2022; Shin, Lee, 2021). Secondly, insect oil, derived from insects such as BSF, can be a valuable source of essential fatty acids, which are important for shrimp nutrition and health (Benzertiha, Kierończyk, Rawski, Mikołajczak, Urbański, Nogowski, Józefiak, 2020; Surendra, Olivier, Tomberlin, Jha, Khanal, 2016).

However, to the best of our knowledge, very few studies have evaluated the health status and histomorphological condition of hepatopancreas of shrimp cultured under commercial condition or in an-outdoor pond until shrimp reaches typical market size for consumption. Therefore, this study was conducted to evaluate the potential uses of BSF meal (BSFM) and BSF oil (BSFO) as the nutrient sources for *L. vannamei* by evaluating growth performance, total haemocyte count, lysozyme activity, and histology of hepatopancreas of shrimp cultured in hapa nets installed within an outdoor pond. In parallel, a separated disease challenge study was also performed to evaluate potential disease resistance of Pacific White leg shrimp fed insect meal and oil.

Materials and Methods

Diet Preparation and Composition

Black soldier fly larvae meal (BSFM) and oil (BSFO) utilized in this experiment were obtained from a local commercial producer (Bio Cycle Indo, Kampar, Riau, Indonesia). The amino acid profile of BSFM and fatty acid profile of BSFO as the interest ingredients are displayed in Table 1. For the experimental diets, ten iso-nitrogenous and iso-lipidic (36% protein and 6% lipid, as fed) diets were prepared at the Main Center for Mariculture Development, Lampung, Indonesia and formulated to meet the specific nutrient requirement for Pacific white shrimp *Litopenaeus vannamei*. Diet 1 (the basal diet) was formulated to be similar to a commercially-available diet in Indonesia. Diet 2 (control diet) was designed to completely replace FO with BSF oil (BSFO) and maintain all the remaining ingredients with similar inclusion level with the basal diet (diet 1). Diets 3 – 6 were formulated to partially replace FM with BSF meal (BSFM) at the inclusion rates of 5, 10, 20 and 50 g Kg^{-1}, respectively and labeled with BSFM 0,5%; 1%, 2% and 5%, respectively. Diets 7 – 10 were formulated to contain with 5 g Kg^{-1} of BSFM to partially replace FM meal as the baseline of the diet and using graded levels of BSFO with inclusion rates of 5, 10, 20 and 42.6 g Kg^{-1} to replace FO, partially and completely, within the diet formulation and labeled with BSFO 0.5; 1; 2 and 5% (Table 2).

Prior to production, all ingredients were crushed and passed through a < 200 mesh sieve (Jinan Shengrun, China), weighed and mixed in a paddle mixer (Marion Mixers, Inc., Marion, IA, USA). The cooking-extrusion temperature was kept at 110°C for approximately 14 seconds in five-barrel sections and the last section was maintained at 62°C. Portion of feeds were extruded into 1- and 2-mm in diameter and 2 – 4 mm in lengths. Diets were air-dried in a pulse bed dryer (Jinan Shengrun, China) and stored at 4°C in sealed containers until further use. The proximate, amino acid, and fatty acid profile of the experimental diets are listed in Table 3 and analyzed at the Saraswanti Indo Genetech Laboratory, Bogor, West Java, Indonesia.

Feeding Trial

The feeding trial was conducted at the Business Service Center for Aquaculture Production (BSCAP), Karawang (West Java, Indonesia) in an outdoor pond. Pacific white shrimp post larvae (PL) were obtained from Salira teknik Benur (Serang, Banten, Indonesia) and acclimatized to the culture system. After 7 days, shrimp (0.97±0.01 g initial mean weight) were then randomly distributed into 40 hapa nets (2 m×2 m×1 m, 200 shrimp per hapa net). The hapa nets were installed in the outdoor ponds, and the source of water was obtained from the surrounding sea-water environment that has been treated and sterilized in a reservoir prior to use for the research. Four replicate groups of shrimp per dietary treatment were administered different types of experimental diets using nutrition research standard protocol for shrimp for 90 days and fed by hand four times daily, at 07:00; 11:00; 15:00 and 20:00 h. The feed inputs were pre-programmed assuming the normal growth of shrimp and feed conversion ratio of 1.5. Daily allowances of feed were adjusted based on observed feed consumption on the feeding trays and weekly sampling of 10 - 15 shrimps per pond which were returned to their ponds after weighing.

Water Quality Analysis and Sample Collection

During the feeding trial, water quality parameters: pH, dissolved oxygen (DO), water temperature, total dissolved solid and salinity were recorded four times daily using sensors (Aqua TROLL 500 Multiparameter Sonde instrument) and the data were stored to an application (AquaEasy apps, Bosch, Singapore) for traceability. Meanwhile, ammonia-nitrogen (NH_3-N),

Table 1. Amino acid profile of black soldier fly *Hermetia illucens* meal (BSFM) and fatty acid profile of black soldier fly *Hermetia illucens* oil (BSFO) used in this study

Amino acid profile of BSFM			
No.	Amino Acid	Unit	Result
1	Aspartic acid	%	4.19
2	Threonine	%	1.47
3	Serine	%	1.54
4	Glutamic acid	%	5.82
5	Glycine	%	2.63
6	Alanine	%	3.60
7	Cystine	%	0.42
8	Valine	%	2.78
9	Methionine	%	0.92
10	Iso-Leucine	%	1.92
11	Leucine	%	3.22
12	Tyrosine	%	2.99
13	Phenylalanine	%	2.06
14	Histidine	%	1.45
15	Lysine	%	2.57
16	Arginine	%	2.58
17	Tryptophan	%	0.74
18	Proline	%	2.88
Total		%	43,77
Protein (N x 6,25)		%	57,22

Fatty acid profile of BSFO			
No.	Fatty Acid	Unit	Result
1	Butyric acid (C4:0)	%	<0.01
2	Caproic acid (C6:0)	%	<0.01
3	Caprylic acid (C8:0)	%	0.06
4	Capric acid (C10:0)	%	1.22
5	Lauric acid (C12:0)	%	24.27
6	Tridecanoic acid (C13:0)	%	0.06
7	Myristic acid (C14:0)	%	15.06
8	Myristoleic acid (C14:1n5)	%	0.19
9	Pentadecanoic acid (C15:0)	%	0.15
10	cis-10-Pentadecanoic acid (C15:1n7)	%	<0.01
11	Palmitic acid (C16:0)	%	18.68
12	Palmitoleic acid (C16:1n7)	%	1.67
13	Margaric acid (C17:0)	%	0.15
14	Magaroleic acid (C17:1n7)	%	0.07
15	Stearic acid (C18:0)	%	2.66
16	Oleic acid (C18:1)	%	24.95
17	Linoleic acid (C18:2)	%	6.93
18	g-Linolenic acid (C18:3n6)	%	<0.01
19	a-Linolenic acid (C18:3n3)	%	0.17
20	Arachidic acid (C20:0)	%	0.12
21	Eicosenoic acid (C20:1n9)	%	0.08
22	Eicosadienoic acid (C20:2n6)	%	0.03
23	Eicosatrienoic acid (C20:3n6)	%	0.02
24	Eicosatrienoic acid (C20:3n3)	%	<0.01
25	Arachidonic acid (C20:4n6)	%	<0.01
26	Eicosapentaenoic acid (C20:5n3)	%	0.07
27	Heneicosanoic acid (C21:0)	%	0.15
28	Behenic acid (C22:0)	%	0.04
29	Erucic acid (C22:1n9)	%	<0.01
30	Docosadienoic acid (C22:2n6)	%	<0.01
31	Docosahexaenoic acid (C22:6n3)	%	0.01
32	Tricosanoic acid (C23:0)	%	0.03
33	Lignoceric acid (C24:0)	%	0.01
34	Nervonic acid (C24:1n9)	%	0.01
Total		%	96,87

nitrate (NO_3-N), nitrite (NO_2-N), and phosphate (PO_4-P) were measured once in a week by using absorption spectrophotometry (DR890, HACH, USA). At the termination of feeding period, the shrimp in each hapa nets were group counted and bulk weighed to calculate the final biomass, final weight, percentage weight gain (PWG), feed conversion ratio (FCR) and percentage survival (SR) according to following equations:

$$\text{PWG}=\frac{(average\ individual\ final\ weight-average\ individual\ initial\ weight)}{(average\ individual\ initial\ weight)}\times 100$$

$$\text{FCR}=\frac{feed\ given\ (g)}{alive\ weigh\ gain\ (g)}$$

$$\text{SR}=\frac{final\ number\ of\ shrimp}{initial\ number\ of\ shrimp}\times 100$$

Total Haemocyte Count

Hemolymph was collected from three individual shrimp per hapa net or twelve shrimp per dietary treatment at the end of the growth trial using a syringe and 25 gauges needle. A 1 mL syringe were preloaded with 0.5 mL of an anti-coagulant and used to collect approximately 0.5 mL of hemolymph from each shrimp. The anticoagulant contains with 30 mM Sodium Citrate Tribasic Dihydrate (Sigma S4641); 0.34 M Sodium chloride (NaCl); and 10 mM EDTA – Ethylene Diamine Tetraacetic Acid (Sigma, E9884) dissolved in distilled water. The hemolymph with anti-coagulant solution were diluted in 150 µL of formaldehyde (4%) and then, 20 µL of the solution were placed on a hemocytometer (Neubauer) to determine THC using an optical microscope (Olympus, DP72).

Lysozyme Activity Analysis

Determination of lysozyme activity level was performed from three individual shrimp per hapa net or twelve shrimp per dietary treatment using commercial kit (Sigma-Aldrich, Cat. no. LY0100), and the methods came from the instruction manual. The results of lysozyme activity were defined by the lysis of the *Micrococcus lysodeikticus* cells. The reactions were conducted at room temperature and with absorbance of 450 nm (Perkin Elmer, Lambda XLS, USA)

$$\text{Lysozyme activity}\ (\frac{\text{Units}}{\text{mL}})=\frac{(\Delta\text{A450}/\min Test-\Delta\text{A450}/\min Blank)\ (df)}{(0.001)(0.03)}$$

df= dilution factor

0.001= ΔA_{450} as per the unit definition
0.03= Volume (in mL) of enzyme solution

Histomorphological Condition of Hepatopancreas and Distal Intestine

The section of hepatopancreas were immediately preserved in Davison's fixative solution for 48 h at room temperature (Bell and Lightner, 1988) and then transferred to 50% ethanol solution (VWR, Radnor, PA, USA) until processed by standard histological analysis procedures. Samples were dehydrated through a standard ethanol series to 100%, embedded in paraffin wax, and sectioned at 4 µm intervals for staining with Hematoxylin-Eosin (H&E) stain (Merck, Darmstadt, Germany). For estimations, double blinded evaluation with a grading scale of 1 to 5 was used. Score 1 was considered as the normal condition and subsequent

Table 2. Ingredient composition (g Kg^{-1}) of experimental diets containing several inclusion levels of insect meal and oil

Ingredients	Experimental Diet Code									
	Basal	BSFO	BSFM 0.5%	BSFM 1%	BSFM 2%	BSFM 5%	BSFO 0.5%	**BSFO 1%**	**BSFO 2%**	**BSFO 5%**
Fish meal[1]	200.00	200.00	193.50	187.00	175.50	144.00	193.50	193.50	193.50	193.50
Soybean meal[1]	372.00	372.00	372.00	372.00	372.00	372.00	372.00	372.00	372.00	372.00
Wheat flour[2]	324.90	324.90	327.70	330.50	333.30	336.10	327.70	327.70	327.70	327.70
Insect meal [3]	0.00	0.00	5.00	10.00	20.00	50.00	5.00	5.00	5.00	5.00
Fish oil[1]	43.90	0.00	42.60	41.30	40.00	38.70	37.60	32.60	22.60	0.00
Insect oil[3]	0.00	43.90	0.00	0.00	0.00	0.00	5.00	10.00	20.00	42.60
Trace mineral premix[5]	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Vitamin premix[6]	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Choline chloride[4]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Stay C[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Soy-Lecithin[4]	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Corn starch[1]	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

[1]PT FKS Multi Agro, Tbk, Jakarta, Indonesia
[2] PT Pundi Kencana, Cilegon, Banten, Indonesia
[3] PT Bio Cycle Indo, Kampar, Riau, Indonesia
[4] PT Fenanza Putra Perkasa, Jakarta, Indonesia
[6]Trace mineral premix (g/100 g premix): cobalt chloride, 0,004; cupric sulfate pentahydrate, 0,550; ferrous sulfate, 2,000; magnesium sulfate anhydrous, 13,862; manganese sulfate monohydrate, 0,650; potassium iodide, 0,067; sodium selenite, 0,010; zinc sulfate heptahydrate, 13,193; alpha-cellulose, 69,664,
[7] Vitamin premix (g/kg premix): thiamin·HCL, 4,95; riboflavin, 3,83; pyridoxine·HCL, 4,00; Ca-pantothenate, 10,00; nicotinic acid, 10,00; biotin, 0,50; folic acid, 4,00; cyanocobalamin, 0,05; inositol, 25,00; vitamin A acetate (500,000 IU/g), 0,32; vitamin D3 (1,000,000 IU/g), 80,00; menadione, 0,50; alpha-cellulose, 856,81

Table 3. Proximate, amino acid (AA) and fatty acid (FA) composition (% as is, dry matter basis) of experimental diets utilized in this trial.

Parameter	Unit	Experimental Diet Code									
		Basal	BSFO	BSFM 0.5%	BSFM 1%	BSFM 2%	BSFM 5%	BSFO 0.5%	BSFO 1%	BSFO 2%	BSFO 5%
Proximate analysis											
Ash content	%	7.41	7.24	7.37	7.36	7.31	7.06	7.12	7.19	7.34	7.21
Energy from fat	Kcal/100 g	57.47	60.59	61.72	59.18	62.38	69.26	69.49	69.54	70.21	69.89
Crude Lipid	%	6.39	6.47	6.48	6.42	6.82	6.99	6.91	6.92	6.95	7.16
Water content	%	11.39	11.71	11.28	11.41	11.49	9.52	10.59	10.49	10.19	10.88
Total energy	Kcal/100 g	356.71	358.95	359.64	359.02	401.54	396.16	394.31	397.18	393.13	399.12
Crude Protein	%	36.23	36.39	36.45	36.51	36.55	36.59	36.66	36.97	36.96	36.99
Crude fiber	%	0.989	0.85	1.05	1.04	1.12	1.09	1.84	0.99	0.99	0.99
Saturated Fat	%	2.60	2.93	2.54	2.53	2.53	2.54	2.54	2.55	2.52	2.52
Amino acid profile (%, as is)											
L-Serine	%	1.83	1.84	1.85	1.99	1.92	1.98	2.00	2.35	2.05	1.99
L-Glutamic acid	%	5.31	5.18	5.33	5.32	5.49	5.76	5.19	5.59	5.28	5.16
L-Phenylalanine	%	2.02	2.18	2.17	2.17	2.18	2.19	2.27	2.93	2.77	2.24
L-Isoleusine	%	1.65	1.68	1.61	1.66	1.68	1.64	1.72	1.82	1.64	1.64
L-Valine	%	1.97	2.03	1.97	1.99	1.99	1.97	2.02	2.15	1.99	2.00
L-Alanine	%	1.63	1.65	1.78	1.66	1.70	1.72	1.63	1.79	1.59	1.61
L-Arginine	%	2.39	2.61	2.37	3.33	2.46	2.42	2.69	3.21	2.97	2.69
Glycine	%	2.05	2.31	2.13	2.41	2.39	2.19	2.25	2.58	2.28	2.31
L-Lysine	%	1.96	1.93	1.98	1.98	1.99	1.99	1.82	1.84	1.81	1.84
L-Aspartic acid	%	2.61	2.57	2.60	2.79	2.70	2.84	2.59	2.82	2.65	2.48
L-Leusine	%	2.78	2.81	2.55	2.91	2.91	2.94	2.79	3.09	2.79	2.79
L-Tyrosine	%	0.26	0.30	0.29	0.23	0.20	0.26	0.25	0.32	0.290	0.22
L-Proline	%	1.97	1.99	1.94	1.99	2.10	2.01	1.99	2.15	1.97	1.99
L-Threonine	%	1.58	1.72	1.50	1.89	1.87	1.85	1.76	2.03	1.80	1.74
L-Histidine	%	0.94	0.99	0.97	1.28	1.19	1.29	1.11	1.32	1.24	1.12
L-Tryptophane	%	0.31	0.32	0.31	0.30	0.33	0.33	0.32	0.33	0.31	0.32
L-Cysteine/Cystine	%	1.23	1.18	1.13	1.15	1.35	1.19	1.18	1.14	1.12	1.29
L-Methionine	%	0.63	0.65	0.64	0.63	0.64	0.63	0.61	0.62	0.62	0.63
Fatty acid profile (*as is*)											
Linolenic acid	%	0.11	0.11	0.09	0.10	0.27	0.14	0.10	0.11	0.03	0.09
Linoleic acid	%	0.74	0.76	0.90	0.66	1.91	0.92	0.75	0.79	0.26	1.01
Oleic acid	%	0.49	0.59	1.10	0.49	1.29	0.58	0.62	0.57	0.25	1.12
10:0	%	ND	ND	ND	ND	ND	0.01	0.02	0.01	0.01	0.06
12:0	%	0.05	0.07	0.08	0.09	0.11	0.20	0.49	0.55	0.57	0.83
14:0	%	0.66	0.75	0.83	0.65	1.69	0.78	0.72	0.72	0.25	0.89
15:0	%	0.04	0.05	0.02	0.04	0.10	0.05	0.04	0.04	0.01	0.03
16:0	%	1.41	1.61	1.20	1.39	3.49	1.71	1.36	1.49	0.45	1.31
17:0	%	0.11	0.13	0.04	0.10	0.26	0.13	0.09	0.11	0.03	0.05
18:0	%	0.29	0.34	0.27	0.28	0.71	0.35	0.27	0.29	0.09	0.22
20:0	%	0.02	0.03	0.01	0.02	0.05	0.03	0.02	0.02	0.01	0.02
24:0	%	0.02	0.02	0.01	0.02	0.05	0.02	0.02	0.01	0.01	0.02
n-3	%	1.86	1.83	1.64	1.50	3.94	2.02	1.46	1.48	0.41	0.73
n-6	%	0.92	0.96	0.96	0.83	2.34	1.14	0.90	0.95	0.31	1.08
n-9	%	0.49	0.59	0.55	0.63	1.29	0.58	0.62	0.57	0.25	1.12
22:6n-3 (DHA)	%	0.75	0.65	0.73	0.73	0.85	0.88	0.84	0.82	0.88	0.87
20:5n-3 (EPA)	%	0.87	0.98	0.83	0.89	0.97	0.93	0.93	0.97	0.90	0.97
16:1	%	0.57	0.67	0.78	0.74	1.39	0.64	0.86	0.83	0.83	0.85
17:1	%	0.08	0.09	0.08	0.08	0.19	0.09	0.09	0.09	0.09	0.09
20:1	%	0.06	0.06	0.06	0.05	0.07	0.06	0.04	0.05	0.04	0.05
18:2n-6	%	0.74	0.76	0.90	0.76	1.91	0.92	0.75	0.79	0.96	1.01
18:1n-9	%	0.49	0.59	1.10	1.49	1.29	1.28	0.62	0.57	0.75	0.92
20:5n-3	%	0.87	0.98	0.83	0.89	1.07	1.03	0.73	0.77	0.80	0.78
20:4n-6	%	0.15	0.16	0.15	0.14	0.35	0.18	0.13	0.13	0.14	0.16
20:3n-6	%	0.02	0.02	0.02	0.02	0.03	0.02	0.01	0.02	0.02	0.02
18:3n-6	%	0.02	0.02	0.02	0.02	0.05	0.02	0.03	0.03	0.03	0.03
18:3n-3	%	0.09	0.09	0.08	0.08	0.23	0.12	0.09	0.09	0.03	0.09
Polyunsaturated Fat	%	2.58	2.79	2.61	2.33	3.28	3.19	2.38	2.44	2.72	2.81
Unsaturated Fat	%	3.78	4.19	2.94	2.49	3.29	3.56	3.57	3.67	3.13	3.23
Monounsaturated Fat	%	1.20	1.41	1.34	1.36	2.01	2.37	1.19	1.23	1.41	1.43

* Analysis conducted by the Saraswanti Indo Genetech Laboratory, Bogor, West Java, Indonesia, Website www,siglaboratory,com

scores accounted for increasing levels of histopathological alteration compared to the normal condition. Images were acquired by using a digital imaging microscope (Olympus BX41, Olympus Optical Co., Ltd., Tokyo, Japan).

Challenge Test by Immersion

In parallel with the hapa net study, the healthy shrimp were cultured in aquaria tank facilities (25 shrimp per tank × 4 replicates = 100 shrimps per dietary treatment) and fed with control and experimental diets for 30 days. On the day 30^{th} of the dietary treatment, 20 shrimps were randomly collected from each tank and placed in the new tank for immersion challenge by using *Vibrio harveyi* at dose of 10^5 cell $shrimp^{-1}$. Shrimp in the negative control group were immersed in distilled water. The infected group was monitored and the cumulative mortality (dead and moribund shrimp) was recorded at 96 h post immersion (Amend, 1981).

Statistical Analysis

Growth parameters, total haemocyte counts, lysozyme activity and challenge test were analyzed using regression and one-way analysis of variance (ANOVA) to determine significant differences among treatments followed by Tukey's multiple comparison tests to determine the difference between treatment means among the treatments. Score data for histomorphological condition of the hepatopancreas of shrimp were treated as categorical data, tested for normality and homoscedasticity and subsequently analyzed using linear regression model. All statistical analyses were conducted using SAS system (V9.4. SAS Institute, Cary, NC, USA).

Results

Composition of the Insect-based Ingredients and Experimental Diets

In our study, the sum of the amino acids contained in BSFM is in the level of 43.77% and crude protein around 57.22%. The level of three limiting amino acids, including methionine, lysine and threonine are presence at the level of 0.92; 2.57; and 1.47%, respectively. In addition, the sum of fatty acid of BSFO is in the level of 96,870% with lauric acid (C12:0) and oleic acid (C18:1) become the highest fatty acid with the level of 24.27 and 24.95%, respectively (Table 1). Proximate composition among test diets (Table 3) showed that the crude protein level ranged from 36.23 % in basal diet (0 g Kg^{-1}; BSFM; 200 g Kg^{-1} FM) to 36.99 % (50 g Kg^{-1} BSFM; 144 g Kg^{-1} FM). Interestingly, as the inclusion of BSFM increases from 5 to 50 g Kg^{-1}, the crude protein level also increases from 36.45 to 36.59%. Dietary lipid ranged from approximately 6.39 % in basal diet to 7.16% in BSFO 5% diet (5 g Kg^{-1} BSFL meal; 42.60 g Kg^{-1} BSFO). Crude lipid level of diets formulated with two different animal oil sources, FO and BSFO, were markedly different (Table 3). The basal diet formulated with 43.90 g Kg^{-1} FO had 6.39% crude lipid, while diet formulated with 43.90 Kg^{-1} BSFO contained 6.47% lipid level. The amino acid profiles of diets 3 to 10 were better compared to the basal diet with respect to the inclusion of BSFM and BSFO in the diet. Moreover, progressively larger differences were found in the concentration of lauric acid (C12:0) within the diet formulated with BSFM and BSFO compared to basal diet.

Water Quality Parameters

Water quality parameters recorded in the experimental pond for the duration of the feeding trial were within the acceptable range for optimum growth and survival of Pacific white shrimp *L. vannamei* and averaged: pH (8.17±0.11); dissolved oxygen (7.48±0.45 mg L^{-1}); water temperature (29.85±0.36°C); and salinity (30.01±1.23 ‰), respectively. Additionally, Ammonia (NH_3-N), Nitrite (NO_2-N), Nitrate (NO_3-N) and Phosphate (PO_4-P) were 0.02±0.04 mg L^{-1}; 0.04±0.01 mg L^{-1}; 6.03±0.66 mg $L^{-1;}$ and 0.01±0.01 mg L^{-1}, respectively.

Growth Performance

In terms of growth performance, all parameters were affected by the inclusion of BSFM and BSFO in the diet formulation. For final body weight (FBW), maximum responses in current study were observed when shrimp were fed with 50 g Kg^{-1} BSFM and combination of 5 g Kg^{-1} BSFM and 20 to 50 g Kg^{-1} BSFO. In general, shrimp FBW, biomass, feed conversion ratio (FCR), percentage weight gain (PWG) and average daily growth (ADG) were better as the inclusion of BSFM and BSFO increases in the diet formulation. The lowest survival rate (SR) was found in the group of shrimp fed without any inclusion of BSFM and BSFO compared to other dietary treatment (Table 4).

Total Haemocyte Counts (THC) and Lysozyme Activity

The total haemocyte count (THC) and lysozyme activity of the shrimp at the end of the growth trial can be viewed in Figure 1 and 2, respectively. Shrimp fed diets containing BSFM and BSFO had higher THC compared to the basal diet, with 2 and 5% BSFO having the highest THC number compared to other dietary treatment. The lysozyme activity were higher in shrimp fed with BSFO, 2 and 5% BSFM; and with all BSFO treatments, intermediate with BSFM 1%, and lowest for the basal diet ($P>0.05$).

Histomorphological Condition of Hepatopancreas of Shrimp

Figure 3 shows the histomorphological conditions of the hepatopancreas of Pacific white shrimp after

being fed with different experimental diet for 90 days. The number of vacuoles and haemocyte infiltration was higher in basal diet and fed with SFM. However, the integrity of hepatopancreas slightly better in shrimp fed with BSFO.

Challenge Test

Mortality of shrimp occurred 24 h post-infection with signs of weakness, passive swimming on the surface, poor feeding, reddish-yellow coloration of the hepatopancreas, and reduced growth rate. Shrimp that were fed with BSFM and BSFO had highest cumulative survival rate (%) compared to the control treatment at the end of the challenge test period (Figure 4).

Statistical Analysis

All data were subjected to one-way analysis of variance to test the effects of experimental diets. In cases where significant differences occurred (significance level = 0.05), the Tukey's multiple comparison test was used to compare means. All statistical analyses were conducted using SAS system (V9.4. SAS Institute, Cary, NC, USA). Differences considered significant at a *P*-value of 0.05 with tendencies considered significant or insignificant for confidence interval of 95%. All Errors bars indicate standard deviation.

Discussion

Although many studies have demonstrated the potential of BSF as one of the ingredients in shrimp *Litopenaeus vannamei*, however, most of the research to date were conducted using aquaria style tanks in the controlled environment of a laboratory setting (Chen, Chi, Zhang, Dong, Yang, Liu, Tan, Xie, 2021; Cummins Jr, Rawles, Thompson, Velasquez, Kobayashi, Hager, Webster, 2017; Richardson, Dantas-Lima, Lefranc, Walraven, 2021; Shin, Lee, 2021). The use of different processing techniques to convert BSF into BSF meal

Table 4. Growth performance of pacific white shrimp *Litopenaeus vannamei* (Mean initial weight 0,97±0.01 g) fed experimental diets for 90 d.

	Biomass (g)	FBW (g)	FCR[1]	PWG (%)[2]	SR	ADG (g/d)
Basal	2205.00^{d}	13.73^{c}	1.40^{a}	1477.87^{c}	80.33^{c}	0.1429^{d}
BSFO	2317.50^{cd}	13.72^{c}	1.40^{a}	1476.84^{c}	84.11^{bc}	0.1427^{cd}
BSFM 0.5%	2462.50^{abc}	13.89^{bc}	1.38^{ab}	1496.84^{bc}	88.63^{ab}	0.1447^{bdc}
BSFM 1%	2452.50^{bc}	14.13^{bc}	1.36^{abc}	1523.56^{abc}	86.83^{abc}	0.1473^{abcd}
BSFM 2%	2595.00^{bc}	14.27^{bc}	1.35^{bc}	1540.52^{ab}	90.91^{a}	0.1489^{ab}
BSFM 5%	2620.00^{a}	14.51^{a}	1.33^{c}	1567.24^{a}	90.32^{a}	0.1515^{a}
BSFO 0.5%	2487.00^{ab}	14.17^{bc}	1.36^{abc}	1529.02^{abc}	87.73^{ab}	0.1478^{abcd}
BSFO 1%	2535.00^{ab}	14.29^{ab}	1.35^{bc}	1542.24^{bc}	87.01^{ab}	0.1491^{ab}
BSFO 2%	2609.50^{a}	14.25^{ab}	1.35^{bc}	1537.64^{ab}	91.33^{a}	0.1488^{ab}
BSFO 5%	2618.50^{a}	14.55^{a}	1.32^{c}	1571.84^{a}	90.84^{a}	0.1519^{a}
P-value	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001
PSE[3]	21.49074	0.0638	0.0021	7.3388	0.9687	0.0007

Note: [1]FCR= Feed conversion ratio; [2] PWG = Percentage Weight gain; [3]PSE = Pooled standard error. Values represent the mean of four replicates, Results in the same columns with different superscript letter are significantly different (P<0.05) based on analysis of variance followed by Tukey's multiple comparison test,

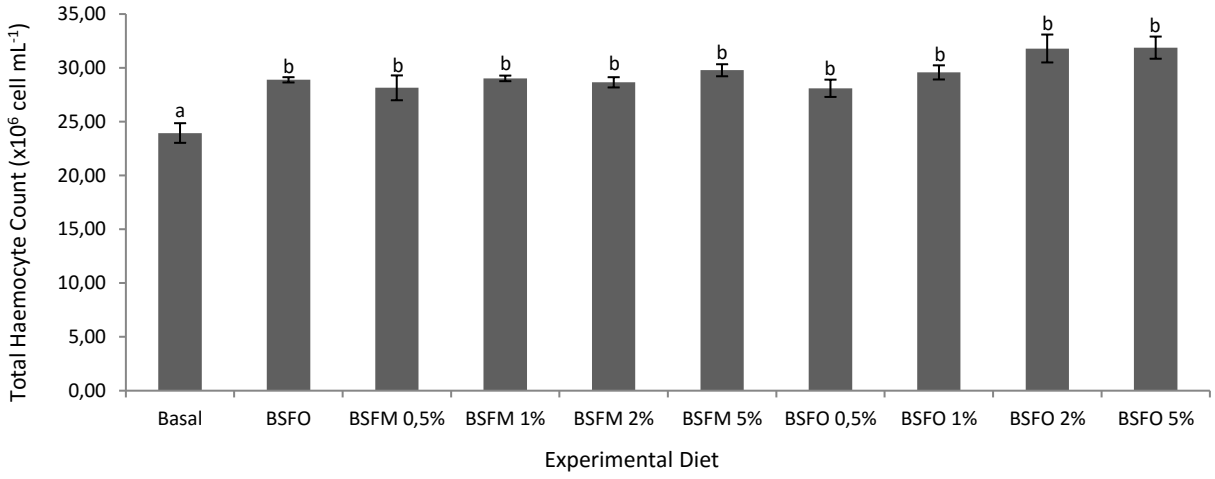

Figure 1, Total hemocyte count of Pacific white shrimp *Litopenaeus vannamei* (10^6 cell mL^{-1}) at the end of feeding trial, Values represent the mean of twelve replicates (P<0.05).

(BSFM) and oil (BSFO) and the utilization of an out-door pond as the culture system may lead to different findings on the growth and health status of shrimp. The present study was the first attempt to determine the effect on the use of BSFM and BSFO obtained from black soldier fly (BSF, *Hermetia illucens*) on the growth and health condition of *Vannamei* cultured using hapa nets installed within an outdoor ponds. In this investigation, the growth of shrimp fed with 0.5 – 5% inclusion levels of BSFM were better compared to the basal diet. Moreover, the utilization of 0.5 – 5% BSFO in combination with 0.5% BSFM significantly generate superior body weight, feed efficiency, percentage weight gain, survival rate and average daily growth compared to the group of shrimp fed with the basal diet. Interestingly, the growth of *L. vannamei* fed with BSFO to completely replace the inclusion of fish oil (FO) within the formulation, gave similar growth with the basal diet fed group. An improved growth performance also found in shrimp fed with graded inclusion levels of BSFM: 4.5; 17.5; and 10.5 g 100 g^{-1}, respectively, to partially replace the inclusion level of FM as much as 30, 50, and 70% over 28 days of feeding period (Richardson, Dantas-Lima, Lefranc, Walraven, 2021). A recent study from Chen, Chi, Zhang, Dong, Yang, Liu, Tan, Xie (2021) affirmed that the inclusion of 4.75; 9.5; and 14.25% to replace 10, 20 and 30% of the FM protein did not affect the growth of shrimp. Similarly, despite BSFM containing higher concentrations of histidine and tyrosine, the studies from Cummins Jr, Rawles, Thompson, Velasquez, Kobayashi, Hager, Webster (2017) showed no significant differences in shrimp growth fed with 7% BSFM compared to the control treatment. Moreover, these authors stated that the growth of shrimp decline with increasing substitution level of BSFM higher than 7% (Cummins Jr, Rawles, Thompson, Velasquez, Kobayashi, Hager, Webster, 2017). The growth reduction with greater inclusion level of BSFM could be a result of the increased amount of chitin that could also affect its nutrient digestibility (Cummins Jr, Rawles, Thompson, Velasquez, Kobayashi, Hager, Webster, 2017). In the present study, focusing on the fulfillment of specific nutrient requirement for shrimp, while considering the level of chitin and anti-nutritional factors (ANFs) from plant-protein source, indicates that a dietary inclusion level of BSFM lower than 5% to replace the inclusion of FM can be used to successfully enhance the growth performance of shrimp. In this capacity, BSFM has some degree of beneficial functionality in this species at this defined level.

With the accelerated growth of shrimp production systems globally, there was an emphasis on the improvement and intensification of production system using ingredients originating from a lower level within the trophic chain (De Silva, Francis, Tacon, 2011). In this respect, the approach was to primarily increase the inclusion of sustainable and economically sound ingredients and reduce the inclusion of FM and FO within the aquafeed formulation (Novriadi, Davis, 2018; Tacon, Metian, 2008). Studies evaluating BSFO as promising feed ingredients in shrimp diets are few but growing at a fast pace. In our research, an attempt to completely replace the inclusion of FO with BSFO did not yield any significant improvement. However, in combination with 0.5% BSFM, the inclusions of BSFO from 0.5 to 5% provide superior biomass and growth performance over 90 d of feeding period. BSFO as the by-product of BSFM production is known to contain high level of medium-chain fatty acid, especially lauric acid (21.4% - 49.3%) (Li, Ji, Zhang, Tian, Zhou, Yu, 2016), which has functional role to reduce the abdominal fat deposition due to their preferential use as an energy substrate over long-chain saturated or unsaturated fatty acid (Kim, Kim, Jeong, Lee, Kim, Lee, Lee, 2020; Li, Ji, Zhang, Tian, Zhou, Yu, 2016; Wang, Wang, Li, Chen,

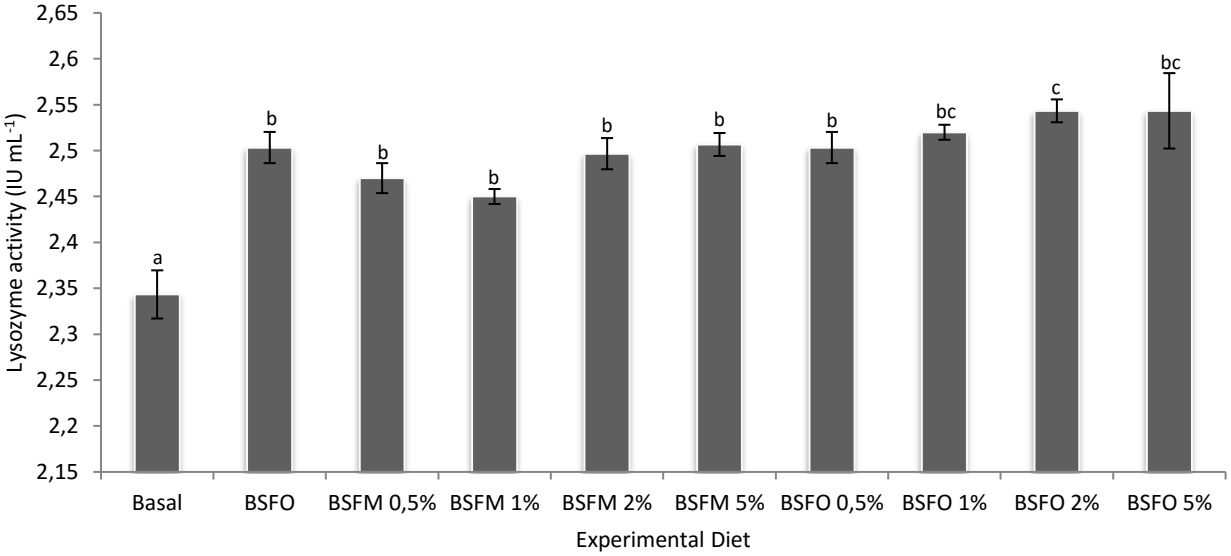

Figure 2, Lysozyme activity of Pacific white shrimp *Litopenaeus Vannamei* (U mL^{-1}) at the end of feeding trial, Values represent the mean of eight replicates ($P<0.05$).

Yang, Zhang, 2015). Moreover, BSFO is also confirmed to be a good source of lipids (more than 28%) and minerals, such as Ca and P (Makkar, Tran, Heuzé, Ankers, 2014; Wang, Shelomi, 2017). Substitution of FO with other alternative lipid source in aquafeed has been established by many workers to change the fatty acid composition of fish and quality of the fillet (Bell, McGhee, Campbell, Sargent, 2003; Davies, Roderick, Brudenell-Bruce, Bavington, Hartnett, Hyland, de Souza Valente, Wan, 2022; Hixson, Parrish, Anderson, 2014). In the current study, it was evident that the growth was comparable between shrimp fed with FO compared to BSFO-based diet. Thus, it can be inferred that the inclusion level of BSFO by as much as 5% to completely replace FO was not beyond the tolerance level of shrimp. Despite the level of 22:6n-3 (DHA) within the BSFO diet being lower compared to FO-based diet, other fatty acid (FA) composition mirrored the profile of FO

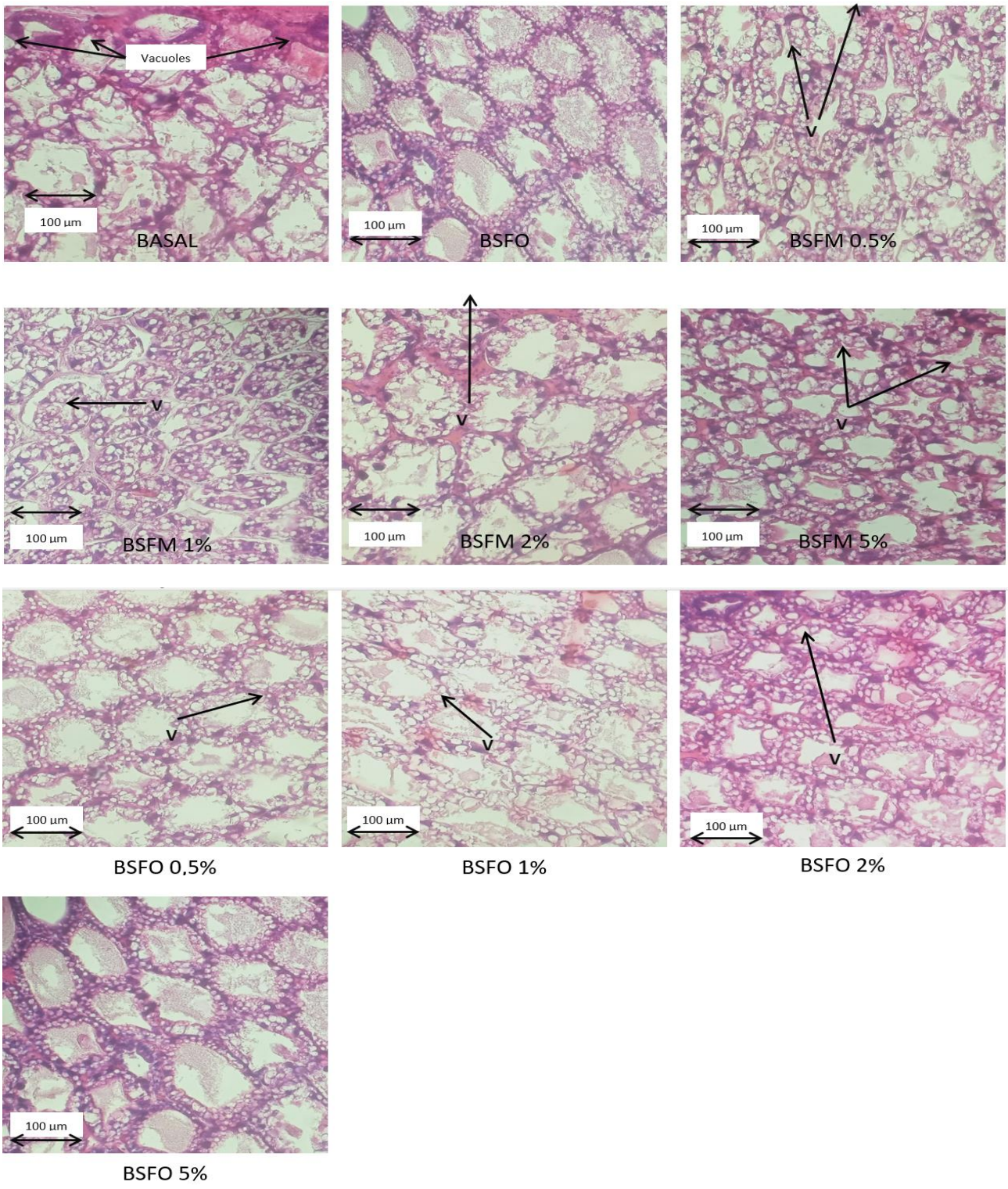

Figure 3, Representative histopathological images of hematoxylin and eosin-stained sections of hepatopancreas of shrimp after feeding with the experimental diets for 90-day.

and whilst still meet the nutritional requirement of shrimp.

FA composition of the BSF larvae depends on the characteristics of the diet (Kim, Kim, Jeong, Lee, Kim, Lee, Lee, 2020). This research using BSF fed on palm kernel meal may be considered as an oil-rich food substrate for bioconversion. In this experimental study, the final product definition of BSFO used contain with LA (C12:0); EPA (C20:5n3) and DHA (C22:6n3) and as much as 24.270%; 0.074% and 0.011%, respectively (Table 1). In this study, the inclusion of 0.5% BSFM in combination with FM and FO, resulted in optimal growth of shrimp fed with graded levels of BSFO ranging from 0.5% to 5%. This blending strategy provides better nutritional profile of the diet that can also support the optimization of shrimp growth. The differences noticed in crude protein, fat, and total energy of the diet as one factor that affect the feed intake (Table 3). A comprehensive review from English, Wanger, Colombo (2021) mentioned that from numerous feeding trials with BSFO in relation to the inclusion rate of BSFM provides synergistic effect to encourage the better growth in salmonid fish mainly.

Further evaluation to observe the functional effect on the use of BSFM and BSFO in shrimp could focus on the structure of the histopathological status of shrimp, as the most reactive organs to the changes of ingredients within the feed formulation (Chen, Zhuang, Yin, Chen, Zhang, Tian, Niu, Liu, 2019; Shao, Wang, Shao, Liu, 2020) and as the main organ for absorption and storage of nutrients (Cervellione, McGurk, Berger Eriksen, Van Den Broeck, 2017; Zhang, Yang, Yan, Zhang, Ye, Sun, 2020). In this study, our research group did not see a systematic interpretable change on the histomorphological condition of the hepatopancreas of the shrimp fed with all dietary treatments. All treatments showed numbers of vacuoles in tubular hepatopancreas and alterations in the star shape of the lumen due to the changes of the tubular epithelial cells. Important to note that the feed provided in this study were using high inclusion level of soybean meal (SBM) as much as 37.2%, moderate level of FM and low amount of BSFM and BSFO. Although SBM is a good sources of alternative protein to substitute the use of animal meal, wider use of SBM may be hindered by negative effects associated with the presence of anti-nutritional factors (ANFs), such as lectins, phytic acid, saponins, phytosterols, and allergens (NRC, 2011), which is responsible for damaging the intestinal health (Bonaldo, Roem, Fagioli, Pecchini, Cipollini, Gatta, 2008; Nordrum, Bakke-McKellep, Krogdahl, Buddington, 2000). A study from Yao, Zhang, Li, He, Wang, Leng (2020) revealed that as the inclusion of SBM increase to replace 17; 33; and 50% dietary FM, the villus height and the intestinal wall thickness of *L. vannamei* decreased significantly. In addition, Rahimnejad, Yuan, Wang, Lu, Song, Zhang (2018) suggest that incorporating high level of SBM in diets for Pacific white shrimp adversely affects gut morphology by lowering the intestinal fold height and a degraded structure compared to the shrimp fed with higher inclusion levels of FM. In the current study, since the changes in the hepatopancreas of the shrimp are not directly related to the growth, indicating that the proper blending strategy with the insect based-nutrients still fulfilled the specific nutrient requirement of the shrimp. Future investigations need to observe the changes in hepatopancreas of shrimp over time as the effect on the escalating use of insect-based proteins to substitute the use of plant-protein sources.

Other than growth and absorption conditions, there is also interest in identifying factors that might be affected because of using novel ingredients, including the digestive status, improving the immunity and resistance to the pathogen. In the present study, since insects are also rich in bioactive compounds, such as chitin, antimicrobial peptides or specific fatty acid, we also evaluate the effect on the use of BSF to the total haemocyte as one of the defense mechanisms to eliminate foreign materials in shrimp (Novriadi, Istiqomah, Isnansetyo, Balk, Jolly-Breithaupt, Davies, 2023); lysozyme activity as an anti-bacterial properties (Kaizu, Fagutao, Kondo, Aoki, Hirono, 2011; Karthik, Kamalakannan, Thomas, Sudheer, Singh, Narayanan,

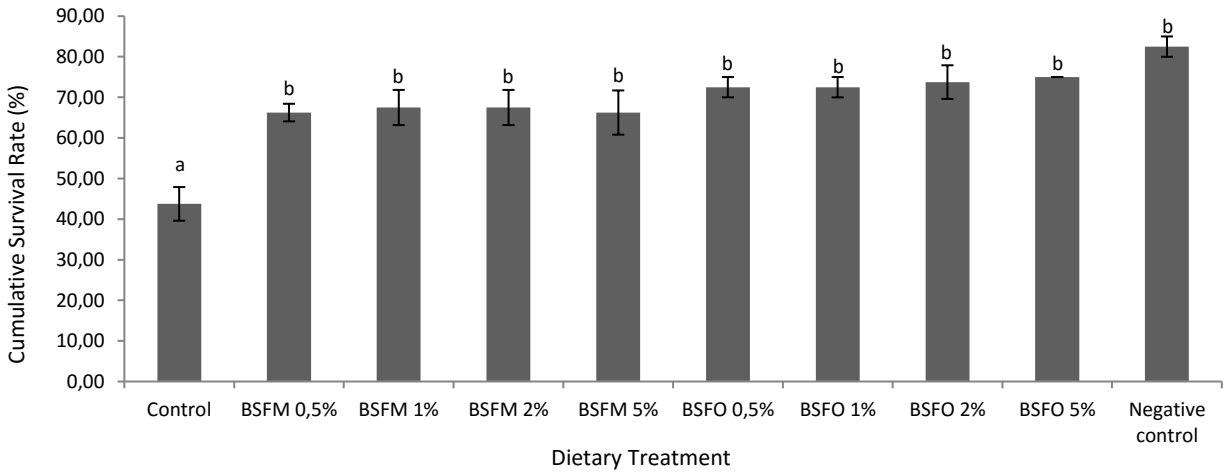

Figure 4, Cumulative mean survival rate (%) of shrimp at the end of challenge test, Different letters indicate statistically significant differences (P<0.05) among the dietary treatment.

2014); survivability of shrimp after challenged with pathogen and histology of the hepatopancreas that allows for the visualization of tissue structure and characteristics changes of the tissue as the results of presenting novel ingredients to shrimp (Gurina, Simms, 2020). In insects, antimicrobial peptides become essential part of the innate immune system and these peptides have antimicrobial abilities when purified from hemolymph (Koutsos, Modica, Freel, 2022). The total haemocyte count (THC, 10^6 cells mL^{-1}) from hemolymph of shrimp in the present study were higher in shrimp fed with BSFM and combination of BSFM and BSFO compared to the basal diet. To date, there is lack of available data on the use of BSFM and BSFO to the THC number in *L. vannamei*. Recent report from Yildirim-Aksoy, Eljack, Beck, Peatman (2022) on the use of BSF frass as by-product of the larval meal industry reveal that the THC number of *L. vannamei* were not significantly affected by the inclusion of 5; 10; 20; and 30 BSF frass meal into the diet. However, research focus on the utilizing of 11 – 12% BSFM to another crustacean, marron (*Cherax cainii*, Austin 2002), into poultry by-product meal (PBM) and FM were able to enhance the THC number. The influence of dietary structural contains within BSFM and BSFO observed in this study reveals the ability of insect-based nutrient to induce the non-specific immune system in shrimp.

BSF-based diets have been shown to reduce the oxidative stress and enhance the antioxidant capacity of the *L. vannamei* (Hu, Wang, Huang, Zhao, Mo, Huang, 2019). In this study, the inclusion of BSFM and in combination with BSFO enhances the lysozyme activity of the shrimp compared to the basal or control treatment. Wang, Peng, Hu, Mo, Wei, Huang (2021) reported that the inclusion of defatted BSFM (DBSFM) to substitute the inclusion of FM as much as 15; 30; 45; 60; and 80% did not alter the lysozyme activity after feeding the shrimp for 56 days using apparent satiation method. Using other species, Hender, Siddik, Howieson, Fotedar (2021) reported that there is no variations of serum lysozyme activity in the juvenile of Asian seabass, *Lates calcarifer* among the dietary treatment, whilst bactericidal activity was significantly increase compared to the control treatment. Interestingly, the supplementation of BSFM to partially replace FM and PBM showed significant enhancement of lysozyme activity in Marron (*Cherax cainaii*) after 60 d of feeding trial (Foysal, Fotedar, Tay, Gupta, 2019). The increase of Lysozyme activity may induce the stimulation of aquatic organisms' immunity system and could contribute to the resistance and survivability against infectious pathogen (Feng, Cai, Chen, Zhu, Chang, Wang, Liu, Zhang, Nie, 2020; Mohammadi, Rafiee, El Basuini, Abdel-Latif, Dawood, 2020).

The induction of the non-specific (innate) immune system plays an important role to enhance the resistance of the shrimp *L. vannamei* to the pathogen invasion (Gui, Mai, Chi, Zhou, Li, Tan, Dong, Yang, Liu, Zhang, 2019). To evaluate the effect on the use of BSFM and BSFO, a challenge test was conducted using *Vibrio harveyi* as one of the important pathogen that responsible for mass mortality in shrimp (Le Groumellec, Haffner, Martin, Martin, 1993; Novriadi, 2016), to compare the resistance of shrimp fed with different inclusion levels of BSFM and BSFO. The outcomes showed that all the shrimp fed with BSFM and BSFO had significantly higher cumulative survival rate compared to the control treatment. Study from Chen, Chi, Zhang, Dong, Yang, Liu, Tan, Xie (2021) demonstrated that the survival rate of shrimp fed with 4.75% BSFM significantly higher compared to the group of shrimp fed with FM after injected with graded levels of *Vibrio parahaemolyticus* for 12 d. Moreover, Richardson, Dantas-Lima, Lefranc, Walraven (2021) mentioned that when challenged with Acute Hepatopancreatic Necrosis Diseases (AHPND) and White Spot Syndrome Virus (WSSV), despite there was an absence of statistical significance, a pattern of high survival for the group of shrimp fed with BSFM was noticed compare to the control treatment when challenged with WSSV. The ability of shrimp treated with insect-based nutrients to protect themselves against pathogenic insult could be due to the potential stimulation of the innate immune system exerted by insect chitin, or due to the presence of lauric acid in the insect meal and oil (Lieberman, Enig, Preuss, 2006). The results of this study demonstrated the benefit of using proper levels of BSFM and BSFO within the diet formulation for shrimp cultured under outdoor intensive pond condition scenarios. Extended research focusing on economic analysis will be warranted to fully assess the overall cost benefit on using BSF-based diet in shrimp industry.

Conclusion

Results showed that 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, health condition, enhance the survival rate after infection with *V. harveyi* at dose of 10^5 CFU mL^{-1}, and maintain the integrity of intestinal histology of the shrimp *L. vannamei.* The total replacement of FO with BSFO did not negatively affect the growth performance of shrimp.

Ethical Statement

All procedures and handling process in the present study were approved by the recommendations in the Guide for the Use of experimental Animals of the Jakarta Technical University of Fisheries, Jakarta, Indonesia

Funding Information

PT. Bio Cycle Indo (Riau, Indonesia) provided the insect meal and oil for the production of experimental diet as well as partial funding for this study.

Author Contribution

Romi Novriadi, Simon Davies, Komang Indra Kurnia Triatmaja: Research conceptualization, Methodology, Validation, formal analysis, investigation, Draft creation, supervision and Writing the original draft; Maman Hermawan, Endhay Kusnendar Muljana Kontara, Budi Tanaka, Ahamd Rinaldy, Jovano Erris Nugroho: Research conceptualization, Methodology, Validation, formal analysis, investigation, and funding acquisition

Conflict of Interest

BT, AR and JEN are employed by PT. Bio Cycle Indo. The rest of the authors state no conflict of interest.

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