Effects of different combination of culture medium on growth and nutrition content of black soldier fly larvae (*Hermetia illuens*)

F Muchdar¹, R Andriani¹, Juharni¹, A Wulansari²

¹Aquaculture Department, Fisheries and Marine Science Faculty, Khairun University, Ternate, Indonesia.
²Food Technology Department, Agriculture Faculty, Khairun University, Ternate, Indonesia.

Email: vina.fisheries@gmail.com

Abstract. Fish feed is the most essential necessity and the biggest contributor on production cost of fish cultivation. As much as 50-70% production cost is for fish feed. It can be deducted by made alternative fish feed. Black soldier fly larvae is an alternative fish feed with high protein content and most importantly it is cheap. The aim of this research was to evaluate the growth and nutrition content of black soldier fly larvae which cultivated in different medium. Medium used in this study was combination of coconut pulp residue and three other different medium, such as tofu dregs, sago dregs, and rice bran. Each combination had three different level of concentration, 1:1, 1:3, 1:5 (w:w). It was evaluated for nutrition content (proximate analysis), biomass, and dried yield. The result showed combination of coconut pulp and sago dregs 1:5 produced the highest biomass (465 g), but black soldier fly larvae produced from combination of coconut pulp residue and tofu dregs 1:3 had the highest protein content (39.78%). The best medium to produce black soldier fly larvae based on multiple attribute decision making (MADM) method was from the combination of coconut pulp residue and sago dregs 1:5 (w:w). It had 465 g biomass, 186 g dried yield, 23.73% protein, 47.67% fat, and 22.15% carbohydrate.

1. Introduction

Feed with high nutritional value is essential for growth, disease resistance, and healthy product of fisheries [1]. The ingredient commonly used in the making of feed are consisted of cereals and cereals by product, animal fats, and vegetable oil as the source of energy; soybean, fishmeal, and meat as the source of protein; mineral supplements such as calcium, sodium; and miscellaneous [2]. Feed with high protein content is well known to be expensive and limited. To comply the high demand of feed with good quality ingredient, many research conducted to find alternative source of ingredient for feed with high nutrient content especially protein but had a low cost [3].

The novel source of nutrition especially protein is from insect. Insect reported has high nutrition value and cheap. It used to be the alternative source of protein to substitute fishmeal and soy bean as the ingredient for animal feed [4]. Black soldier fly larvae (BSFL) is a famous feed ingredient. BSFL contained of 42% crude protein and 29% fat [5]. Study by [6] showed, BSFL had 399-431 g/kg crude protein and 27-197 g/kg crude ash. It was rich in lysine, valine, and arginine. It also was rich in calcium and kalium.

BSFL is recommended as ingredient of animal feed. It can be grown easily and does not need special treatment or facilities. [7] reported fish (European seabass) fed with 19.5% BSFL had the same
growth performance as fish fed with fish meal. Another research conducted by [8] reported fish fed by 50% BSFL had the highest body weight, weight gain, and specific growth rate. The fish fed by BSFL also had higher whole-body protein while the fat and ash contents were not significantly different with fish fed with 100% fish meal.

BSFL cultivate in various culture medium. Culture medium that widely used to grow BSFL are chicken feed, vegetables waste, fruits (apple, pear, orange) substrate, and vegetables (lettuce, green beans, and cabbage) substrate [9, 10]. Some other research conducted to find alternative culture medium to produce BSFL, such as palm kernel cake, rice straw, soy bean curd residue, and coffee silver skin [11], [12], [13], [14]. All medium above were waste product of agriculture. Some waste products may need pretreatment or addition of another ingredient such as battery and microalgae before it can be used as culture medium of BSFL.

Coconut pulp residue, tofu dregs, sago dregs, and rice bran are waste product which are abundant in North Maluku, Indonesia. Coconut pulp residue is by-product of coconut after it was extracted of milk and oil. It consisted of 1.69% protein; 17.26% fat; 25.73% carbohydrate; 0.54% ash; and 55.42% water [15]. Tofu dregs is a by-product of tofu industry. Dried tofu dregs consisted of 18.68% carbohydrate; 0.57% fat; 12.77% protein; 0.35% ash; and 7.63% water [16]. Meanwhile sago dregs is a waste product of sago industry. Sago dregs is high in carbohydrate and fiber. [17] reported, sago dregs consisted of 82.4% carbohydrate; 0.80% protein, 0.01% fat; 0.50% ash; 1.67% crude fiber; and 16.3% water. Rice bran is the layer covered rice kernel. It has brown color. It also has high nutrition component such as dietary fiber, protein, fat, minerals (K, Ca, Mg, Fe), and antioxidant (tocopherol, tocotrienols, γ-oryzanol) [18]. Based on the previous research, coconut pulp residue, tofu dregs, sago dregs, and rice bran are rich in nutrient. It should be utilized more rather than being a waste only.

BSFL cultivation is quite easy. It can grow in many kind of organic waste. The production is higher in plant substrate than animal substrate. The production cost is also low, but BSFL is rich in protein. It can be potential alternative protein source for animal feed. Coconut pulp residue, tofu dregs, sago dregs, and rice bran with high nutrition content, have the potential to be medium to cultivate BSFL.

2. Material and methods

2.1 Cultivation of BSFL

Coconut pulp residue was kindly obtained from local coconut farmer. A tofu dreg was obtained from local tofu industry. A sago dreg was obtained from local sago home industry, while rice bran was obtained from local rice farmer in Ternate, North Maluku Indonesia.

The culture medium was made from the combination of coconut pulp residue (CP) and one of another three main ingredient tofu dregs (TD), sago dregs (SG), and rice bran (RB). All main ingredients were dried under the sun for 3 days. There were three different proportions of coconut pulp residue and another ingredient, 1:1, 1:3, 1:5. There were 9 groups of culture medium with different combination and every group was repeated for 3 times. Another ingredient added to the mixture were water, Lactobacillus casei and chicken stock powder.

The container for cultivation of BSFL was made from plastic basin which covered up with banana leave and net wire. The container was kept in a shady place, humid, and the temperature was around 27-32°C. The temperature was checked every day. Every two days banana leave was changed to the new one.

Black soldier fly was spotted on the medium and around 2 days small BSFL was spotted on the banana leave. The cultivation was last for 3 weeks. BSFL was harvested after 3 weeks cultivation. Biomass of BSFL was determined by weighed the BSFL directly after it was harvested. BSFL then dried under the sun for 3 days. Finally, the dried BSFL was weighted.

2.2 Proximate analysis

Proximate analysis was conducted based on method by [19]. The BSFL was dried under the sun for 3 days. Dried BSFL then analyzed for water content (thermogravimetric method), ash (drying-ash
method), crude protein (Kjeldahl method), fat (Soxhletation method), crude fiber (Soxhletation method), and carbohydrate. The analysis was repeated for 3 times for every sample.

3. Results

Cultivation period was last for 3 weeks. The temperature during cultivation was around 27-36ºC. BSFL cultivated in combination of coconut pulp residue and rice bran 1:3 (CPRB3) and 1:5 (CPRB5) had the highest temperature (36ºC) which may cause none BSFL was found in both groups. Culture medium made of combination coconut pulp residue and tofu dregs 1:5 had the lowest temperature among all treatment groups (Table 1).

Table 1 Cultivation temperature, biomass, and dried weight of black soldier fly larvae cultivated in different culture medium

| Groups     | Cultivation Temperature (ºC) | Biomass (g) | Dried Matter (g) |
|------------|------------------------------|-------------|------------------|
| CPTD1      | 28                           | 100.0       | 40.0             |
| CPTD3      | 28                           | 137.5       | 55.0             |
| CPTD5      | 27                           | 177.5       | 71.0             |
| CPSD1      | 32                           | 185.0       | 74.0             |
| CPSD3      | 32                           | 307.5       | 123.0            |
| CPSD5      | 30                           | 465.0       | 186.0            |
| CPRB1      | 34                           | 210.0       | 84.0             |
| CPRB3      | 36                           | 0.0         | 0.0              |
| CPRB5      | 36                           | 0.0         | 0.0              |

CPTD1= coconut pulp residue: tofu dregs (1:1); CPTD3= coconut pulp residue: tofu dregs (1:3); CPTD5= coconut pulp residue: tofu dregs (1:5); CPSD1= coconut pulp residue: sago dregs (1:1); CPSD3= coconut pulp residue: sago dregs (1:3); CPSD5= coconut pulp residue: sago dregs (1:5); CPRB1= coconut pulp residue: rice bran (1:1); CPRB3= coconut pulp residue: rice bran (1:3); CPRB5= coconut pulp residue: rice bran (1:5)

Biomass was determined by weighed the BSFL directly after harvested. BSFL was separated from the remain medium, washed, and weighed. Culture medium made from coconut pulp residue and sago dregs 1:5 (CPSD5) had the highest production of BSFL. It could produce 465 g BSFL. The lowest group was BSFL cultured in combination of coconut pulp residue and tofu dregs 1:1 (CPTD1), which produced 100 g BSFL (Table 1). BSFL cultivated in medium made from coconut pulp residue and sago dregs (CPSD) in all levels of concentration had the highest biomass than BSFL cultivated in medium made from coconut pulp residue and tofu dregs (CPTD). Culture medium made from coconut pulp residue and rice bran 1:1 (CPRB1) had the highest among another group with the same level of concentration, but unfortunately BSFL could not be found in another group of CPTD.

BSFL was dried under the sun for 3 days. Dried BSFL then weighed to determine the yield. Dried BSFL was linear with the number of biomass. CPSD5 was the highest production of BSFL which led to high biomass and dried BSFL. Whereas CPTD1 had the lowest biomass number which led to lowest yield of BSFL (Table 1).

3.1 Nutrition component

Dried BSFL was tested for nutrition component using proximate analysis. Water content was analyzed using thermogravimetric method and every sample was repeated for 3 times. BSFL harvested from medium made of coconut pulp residue and tofu dregs 1:5 (CPTD5) had the highest water content (14.11%), while BSFL from the combination of coconut pulp residue and sago dregs 1:5 had the lowest water content (6.63%). The average of BSFL water content was 6.63-14.11% (Table 2).
Humidity that is required to grow BSFL is >65%.[20] Based on the result BSFL could survive in temperature 27–30ºC. [20] reported 74-97% BSFL could survive in temperature 27-30ºC and only 0.1% BSFL cannot survive in temperature 36ºC. Humidity that is required to grow BSFL is >65% [21]. In this study, the temperature where BSFL grew using medium made from coconut pulp residue and rice bran 1:3 (CPRB3) and 1:5 (CPRB5) was 36ºC. It was too high for the larvae to survive. It caused no larvae was found after 3 weeks of cultivation. Based on the result BSFL could survive in the temperature range of 27-34ºC.

### Table 2. Nutrition composition of dried black soldier fly larvae

| Groups       | Water (%) | Crude Protein (%) | Crude Fat (%) | Crude Fiber (%) | Carbohydrate (%) | Ash (%) |
|--------------|-----------|-------------------|---------------|-----------------|------------------|--------|
| CPTD1        | 10.80     | 27.91             | 43.17         | 10.75           | 24.30            | 4.63   |
| CPTD3        | 12.12     | 39.78             | 40.90         | 10.82           | 14.67            | 4.64   |
| CPTD5        | 14.11     | 39.10             | 36.87         | 9.52            | 17.90            | 6.63   |
| CPSD1        | 10.35     | 37.31             | 36.98         | 9.66            | 20.44            | 5.27   |
| CPSD3        | 7.65      | 26.03             | 41.13         | 9.93            | 26.06            | 6.77   |
| CPSD5        | 6.63      | 23.73             | 47.67         | 9.17            | 22.15            | 6.45   |
| CPRB1        | 7.99      | 36.59             | 44.16         | 9.40            | 21.61            | 7.65   |
| CPRB3        | 0.00      | 0.00              | 0.00          | 0.00            | 0.00             | 0.00   |
| CPRB5        | 0.00      | 0.00              | 0.00          | 0.00            | 0.00             | 0.00   |

CPTD1= coconut pulp residue: tofu dregs (1:1); CPTD3= coconut pulp residue: tofu dregs (1:3); CPTD5= coconut pulp residue: tofu dregs (1:5); CPSD1= coconut pulp residue: sago dregs (1:3); CPSD3= coconut pulp residue: sago dregs (1:5); CPRB1= coconut pulp residue: rice bran (1:1); CPRB3= coconut pulp residue: rice bran (1:3); CPRB5= coconut pulp residue: rice bran (1:5).

Crude protein of BSFL was around 23.73-39.78%. BSFL cultivated in medium made from coconut pulp residue and tofu dregs 1:3 (CPTD3) had the highest crude protein content, while medium made from coconut pulp residue and sago dregs 1:5 (CPSD5) has the lowest protein content. CPSD5 medium produced BSFL with the highest crude fat content (47.67%) and the lowest one was BSFL from coconut pulp residue and tofu dregs 1:5 combination (36.87%).

Crude fiber of BSFL was between 9.17 and 10.82%. BSFL cultivated from CPTD3 medium had the highest crude fiber content, and the lowest was BSFL from CPSD5. Culture medium made from coconut pulp residue and sago dregs 1:3 (CPSD3) produced BSFL with the highest carbohydrate content (26.06%) and the lowest one was BSFL cultivated using medium made from CPTD3 (14.67%). Ash content was represented mineral content in BSFL. BSFL cultivated in medium made from coconut pulp residue and rice bran 1:1 (CPRB1) had the highest ash content (7.65%). The lowest ash content was BSFL produced by medium made from coconut pulp residue and tofu dregs 1:1 (CPTD1) (Table 2).

#### 3.2 The best culture medium

The best culture medium was determined using multiple attribute decision making (MADM) method by [24]. The result showed medium made from coconut pulp residue and sago dregs 1:5 (CPSD5) was the best medium to produce black soldier fly larvae. CPSD5 could produce 465 g fresh BSFL and 186 g dried BSFL. It was the highest among all groups. The nutrition content of BSFL harvested from it had 6.63% water content, the lowest one; 23.73% crude protein; 47.67% crude fat, the highest among all; 9.17% crude fiber; 22.15% carbohydrate; and 6.45% ash.

#### 4. Discussion

The larvae of black soldier fly can consume any kind of organic substrate, which mostly is a waste product. Temperature and humidity are two important factors for BSFL to live other than the medium. The temperature suitable for BSFL to live and grow is around 27-30ºC. [20] reported 74-97% BSFL could survive in temperature 27-30ºC and only 0.1% BSFL cannot survive in temperature 36ºC. Humidity that is required to grow BSFL is >65% [21]. In this study, the temperature where BSFL grew using medium made from coconut pulp residue and rice bran 1:3 (CPRB3) and 1:5 (CPRB5) was 36ºC. It was too high for the larvae to survive. It caused no larvae was found after 3 weeks of cultivation. Based on the result BSFL could survive in the temperature range of 27-34ºC.
After a female black soldier fly laying the eggs, they need 4-5 days to turn to larvae. The larva needs at least 2 weeks to turn to be mature [21]. During the maturing period, larvae density and medium nutrient are important matters. The concentration between coconut pulp residue and tofu dregs or sago dregs affected the production of BSFL. Increasing the concentration of tofu dregs or sago dregs to three and five times than coconut pulp residue could produce more BSFL than the same concentration among each ingredient. [22] reported increasing nutrient value of culture medium affected weight of individual larvae and larvae yield. This study result showed medium made of coconut pulp residue and tofu dregs 1:5 (CPTD5) had the highest yield or dried matter than other same medium with lower concentration of tofu dregs. It also applied for group of medium made from coconut pulp residue and sago dregs.

Nutrition composition of the culture medium may affect the growth of BSFL. Tofu dregs and rice bran are rich in protein. [16] reported tofu dregs consisted of 12.77% crude protein. While sago dregs had 0.8% crude protein content [17]. Sago dregs had much lower protein content than tofu dregs. The BSFL produced by medium made from coconut pulp residue and tofu dregs had more protein content than BSFL produced by medium made from coconut pulp residue and sago dregs. Rice bran had slightly higher protein than tofu dregs. [23] reported rice bran crude protein content was around 12.26-14.01%. Protein content of BSFL produced by medium from coconut pulp residue and rice bran 1:1 (CPRB1) had the highest protein content than the other groups in the same concentration. Based on this result, protein content of culture medium may affect the protein content of BSFL cultivate in it.

BSFL cultivated in media made from coconut pulp residue and sago dregs 1:5 (CPSD5) had the highest crude fat. In average, BSFL produced by CPSD culture medium had higher fat and carbohydrate content than the other groups. CPSD culture medium produced BSFL with crude fat content around 36.98-47.67% and carbohydrate around 20.44-26.06%. It was higher than BSFL produced by medium made from coconut pulp residue and tofu dregs (CPTD). Sago dregs had the highest carbohydrate content than tofu dregs and rice band. The carbohydrate content of sago dregs, rice bran, and tofu dregs from the previous studies, sequentially was 82.4%; 42.19-45.74%; and 18.68% [17], [16], [23]. Sago dregs high carbohydrate content may affect BSFL’s carbohydrate and fat contents.

The best culture medium was determined using multiple attribute decision making (MADM) method by [24]. It was evaluated from biomass, yield, and nutrition components. The result showed culture medium made from combination of coconut pulp residue and sago dregs 1:5 (CPSD5) was the best culture medium to produce BSFL. It was produced the highest yield among all. The nutrition composition of BSFL cultivated in medium CPSD5 was 6.63% water; 23.73% crude protein; 47.67% crude fat; 22.15% carbohydrate; 9.17% crude fiber; and 6.45% ash.

5. Conclusions
BSFL can grow in all media made from organic waste as long as the culture place condition are well maintained. Studies reviewed indicated that temperature range suitable for BSFL to grow was 27-34°C. Nutrition composition of culture medium was also one of main factor that affected the growth of BSFL. Nutrition composition of BSFL would depend on the nutrition composition of the medium. Coconut pulp residue, tofu dregs, sago dregs, and rice bran are by product of agricultural industry that still has nutrition component and mostly will end up as a trash. Turning the waste as medium of BSFL could be a solution to utilize it and produce BSFL as alternative fish feed.

Acknowledgements
The author would like to thank the Chancellor of Khairun University, the Dean of the Faculty of Fisheries and Marine Affairs of the Khairun University and the students who helped that made this research could be completed properly.
References

[1] Bhosale S V, Bhilave M P, Nadaf S B 2010 Formulation of fish feed using ingredients from plant sources. *Journal of Agriculture Sciences*. 3284-287.

[2] Revindran V 2013 *Main ingredient used in poultry feed formulation*. In: Poultry development review. FAO (Eds.). (New Zealand: Palmerston North) 67-69.

[3] Kim S W, Less J F, Wang L, Yan T, Kiron V, Kaushik S J, Lei X G 2019 Meeting global feed protein demand: challenge, opportunity, and strategy. *Annual Review of Animal Biosciences* (AroAB) 7221-243.

[4] El-Sayed A F M 2020 *Tilapia Culture*. Academic Press, Cambridge. (Ingrissi: Elsevier) pp. 135-172.

[5] Wang Y and Shelomi M 2017 Review of black soldier fly (*Hermetia illucens*) as animal feed and human food. *National Library of Medicine*. 6(10) 91.

[6] Spranghers T, Ottoboni M, Klootwijk C, Ovyn A, Deboosere S, Meulenaer B D, Michiels J, Eeckhout M, Clercq P D, Mert S D 2016 Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. *Journal of the Science of Food and Agriculture*. 97(8) 2594-2600.

[7] Magalhaes R, Sanchez-Lopez A, Leal R S, Martinez-Llorenz S, Olivia-Teles A, Peres H 2017 Black soldier fly (*Hermetia illucens*) pre-pupa meal as a fish meal replacement in diets for European seabass (*Dicentrarchus labrax*). *Aquaculture* 476 79-85.

[8] Fawole F J, Adeeoye A A, Tamiyu L O, Ajala K I, Obadara S O, Ganiyu I O, 2020 Substituting fishmeal with *Hermetia illucens* in the diets of African catfish (*Clarias garupinus*): Effect on growth, nutrient utilization, haematophysiological response, and oxidative stress biomarker. *Aquaculture* 518 734-849.

[9] Boccazzi I V, Ottoboni M, Martin E, Comandatore F, Vallone L, Spranghers T, Eeckhout M, Mereghetti V, Pinotti L, 2017 A survey of the mycobiota associate with larvae of the black soldier fly (*Hermetia illucens*) reared for feed production. *Animal* 12(8) e0182533.

[10] Jucker C, Erba D, Leonardi M G, Lupi D, Savoldelli S 2017 Assessment of vegetable and fruit substrates as potential rearing media for *Hermetia illucens* (Diptera: Stratiomyidae) larvae. *Environmental Entomology* 46(6) 1415-1423.

[11] Bukau R J M and Witoko P 2017 Optimization of bioconversion process of palm kernel cake for production maggot *Hermetia illucens* as a source of animal protein in fish farming. *Aquaculture Indonesia* 18(1) 20-25.

[12] Manurung R, Supriatna A, Esyanthi R R, Putra R E, 2016 Bioconversion of rice straw waste by black soldier fly larvae (*Hermetia illucens*:): optional feeding rate for biomass production. *Journal of Entomol Zool Stud* 4(4) 1036-1041.

[13] Somroo A A, Rehman K, Zheng L, Dai M, Xiao X, Hu S, Mathys A, Gold M, Yu Z, Zhang J, 2019 Influence of *Lactobacillus buchneri* on soybean curd residue co-conversion by black soldier fly larvae (*Hermetia illucens*): food and feedstock production. *Waste Management* 86 114-122.

[14] Truzzi C, Giorgini E, Annibaldi A, Antonucci M, Illuminati S, Scarponi G, Riolo P, Isidoru N, Conti C, Zarantoniello M, Cipriani R, Olivotto I 2020 Fatty acids profile of black soldier fly (*Hermetia illucens*): influence of feeding substrate based on coffee waste silver skin enriched with microalgae. *Animal Feed Science and Technology* 259 114309.

[15] Nor N N M, Abbasi Sadi S, Mariakk M N, Ariff A, Amid M, Lamhusdin D U, Manap M Y A, Mustafa S 2017 Defatted coconut residue crude polysaccharides as potential prebiotics: study of their effects on proliferation and acidifying activity of probiotics in vitro. *Journal of Food Science and Technology* 54 164-173.

[16] Putri E C J and Sumardiono S 2020 Fiber content of analog rice production from composite flour: cassava, avocado seeds, and tofu waste. *Journal of Physics: Conference Series* 1517 012027.
[17] Dhiputra I M K and Jonatan N J 2015 The utilization of metroxylon sago dregs for eco-friendly bioethanol stove in Papua, Indonesia 119-125.
[18] Sharif M K, Sadiq M, Butt M S, Anjum F M, Khan S H, 2014 Rice bran: a novel functional ingredient. Critical Review in Food Science and Nutrition 54 (6) 807-816.
[19] SNI 01-2891-1992., 1992 How to Test Food and Beverages. Industrial Standardization Center, Ministry of Industry Jakarta Indonesia.
[20] Tomberlin J K, Adler P H, Myers H M, 2009 Development of the black soldier fly (Diptera: Stratio, yidae) in relation to temperature. Environment Entomology 38 (3) 930-934.
[21] Salomone R, Saija G, Mondello G, Giannetto A, Fasulo S, Savastano D 2017 Environmental impact of food waste bioconversion by insect: application of life cycle assessment to process using Hermetia illucens. Journal of Clear Production 140 (2) 890-905.
[22] Barragan-Fonseca K B, Dicke M, Van Loon J J A 2018 Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black soldier fly larvae (Hermetia illucens). Entomologia Experimentalis et Applicata 166 (90) 761-770.
[23] Satter M A, Ara H, Jabin S A, Abedin N, Azad A K, Hossain A, Ara U 2014 Nutritional composition and stabilization of local variety rice bran BRRI-28. International Journal of Science and Technology 3 (5) 306-313.
[24] Zeleny M 1982 Multiple Criteria Decision Making. Mc Graw Hill, New York.