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OMEGA-3 CONTENT OF BLACK SOLDIER FLY PREPUPA (*Hermetia illucens*) FED WITH MARINE FISH OFFAL AND TOFU DREG

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ABSTRACT

One of the most promising materials with great potential as a nutrition source of animal feed is the black soldier fly prepupae (BSFP) (*Hermetia illucens*) which is fed with organic wastes. This study was designed to observe the accumulation of omega-3 from the organic wastes of marine fish offal and tofu dregs converted as the biomass of the harvested BSFP. A total of 150 g food consisted of marine fish offal and tofu dreg with different proportions of fish offal:tofu dreg at 10:90, 25:75, 50:50, and 100% tofu dregs were fed to black soldier fly larvae (BSFL) for 21 days. At the end of the feeding period, all BSFL were harvested, weighed, and then analyzed for omega-3 fatty acids content using Gas Cromatography with Flame Ionization Detector (GC-FID). Results showed that BSFL reared with 25% marine fish offal produced the highest biomass (8.1 g/50 larvae) with the least development time (19 days). The total omega-3 recorded from the application of 0%, 10%, 25%, and 50% of marine fish offal was 0.02%, 0.87%, 2.16% and 2.61% in 100 g of dry weight, respectively. These results showed the possibility of producing specific nutrients from BSFP biomass converted from organic wastes, thereby providing baseline knowledge for further formulation of specific animal feed from BSFP.

Keywords: black soldier fly, fish offal, omega-3, tofu dregs

INTRODUCTION

Organic waste is the most dominant waste produced in Indonesia (Zulfikar & Chaerul 2010). Most of these end up as piles of wastes as the collection and waste management efforts could only cover about 50 - 70% of wastes produced by the population (Cointreau 1994; Rushbrook & Pugh 1999). Common methods applied to manage these wastes include the costly sanitary landfill and composting which produce compost of low economic value, thereby making both methods less desirable in low income regions (Cointreau 1994).

Another alternative in managing organic waste is its conversion into useful biomass using saprophages through the natural decomposition process (Beard & Sands 1973; Boushy 1991; Barnard *et al.* 1998; Ndegwa & Thompson 2001; Ramos-Elorduy *et al.* 2002; Barry 2004; Diener *et al.* 2009; Manurung *et al.* 2016; Supriyatna *et al.* 2016). Latest developments include the increasingly becoming popular trend of using insect larvae as organic wastes decomposting agents, in producing a protein- and lipid-rich biomass (Diener *et al.* 2009). Among these, the Black Soldier Flies Larvae (BSFL) (*Hermetia illucens*) has been considered to be the best candidate due to their great ability to convert various types of organic wastes into high protein-rich biomass which could be high quality sources of protein and lipid for animal feed, as well as, potential sources of biodiesel (Newton *et al.* 1977; Newton *et al.* 2005a, b; St-Hilaire *et al.* 2007a; Myers *et al.* 2008; Rachmawati *et al.* 2010; Li *et al.* 2011; Muin *et al.*

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2017; Renna *et al.* 2017; Spranghers *et al.* 2017; Kinasih *et al.* 2018).

Other benefits of using *H. illucens* as organic wastes conversion agent, especially in tropical regions like Indonesia, are: 1. the species is adapted to tropic and warm subtropical region (Tomberlin *et al.* 2002); 2. able to suppress the population of house flies (*Musca domestica*) through predation and competition for nutrition (Furman *et al.* 1959; Newton *et al.* 2005a, b); 3. able to reduce pathogens like *Escherichia coli* and *Salmonella enterica* (Erickson *et al.* 2004); 4. has short development time (about 2 to 3 weeks) (Newby 1997); 5. adult flies do not eat fresh material and females do not oviposit their eggs directly on food source of larvae which made them less likely to tranfer disease (Leclercq 1997); and 6. adult flies are not attracted to human habitats or food (van Huis *et al.* 2013). Moreover, this insect has digestive system rich with amylase, lipase, protease, α-galactosidase, β-galactosidase, α-mannosidase, and α-fucosidase allowing them to digest almost any organic materials (Kim *et al.* 2011). Like other insects, their digestive system also allows them to accumulate certain organic molecules (St-Hilaire *et al.* 2007b).

All of these benefits can be harnessed to solve one of the main obstacles of food security in Indonesia, particularly protein insufficiency, or the limited availability of protein rich yet affordable feed stock. Among organic wastes produced in large volumes in Indonesia, wastes with relatively unchanged organic matter properties during its production are tofu dreg and marine fish offal. About 731,501.5 ton of tofu dreg is produced annually (Bisnis UKM 2009), while 25 - 30% of marine fish catch ended up as wastes (KKP 2016). Valuable organic wastes such as tofu dreg is rich in carbohydrate and protein (Li *et al.* 2012; Li *et al.* 2013), while the marine fish tissues are rich in beneficial unsaturated fatty acids (Sahena *et al.* 2009).

The nutritional content of BSFL biomass highly depends on quantity and quality of the feeding material (Nguyen *et al.* 2015; Oonincx *et al.* 2015). Moreover, the fat content showed more variation (6.6 to 39.2%) than protein (37.0 to 62.7%) (Barragan-Fonseca *et al.* 2017) suggesting the strong effect of the larval dietary nutrient to protein and fat content. One main purpose of BSFL production is to manufacture an alternative source of feed material for several livestock, such as poultry (Leiber *et al.* 2017; Ssepuuya *et al.* 2017) and cultured fish species (Webster *et al.* 2015; Stadtlander *et al.* 2017). A growing number of studies showed the possibility of modifying the fatty acid profile of BSFL, improving the fat nutrient value (i.e., healthier fats such as omega-3) of local livestock product through manipulation of feed for better valuation of the product (Stoneham *et al.* 2018).

The lipid content of insects is largely dependent on their diet (Stanley-Samuelson & Dadd 1983) and many of them have natural long-chain unsaturated fatty acids in their biomass (Thompson 1973). These omega-3 content of BSFL could be enhanced by allowing them to feed on fish waste from rainbow trout processing plant (St-Hilare *et al.* 2007b). Unlike rainbow trout, most marine tropical fishes have low omega-3 content. In this study, the combination of both marine fish offal and tofu dreg were utilized as feeding material of BSFL to enhanced the omega-3 content of its biomass. The main objective of this study is to produce BSFL biomass with high omega-3 content at laboratory scale as a basis for large scale production of an Omega-3 rich alternative source of livestock feed converted from organic wastes.

MATERIALS AND METHODS

Animal Specimen

The larvae of the black soldier fly were obtained from the eggs that were purchased from BSF farm in Sumedang, West Java. All eggs were kept in the substance made of commercial chicken feed (60% moisture) and kept at a constant temperature (28 °C, 70% RH) in a container (50 x 25 x 10 cm) at the Laboratory of Enviromental Toxicology, School of Life Sciences and Technology, Bandung, Indonesia.

Animal Treatment

The marine fish offal originally from local fish market and tofu dreg, produced as waste of the local tofu industry of Sumedang, West Java,

Table 1 Proximate analysis of the feeding material

Proportion of fish offal to tofu dreg	Protein (%)	Lipid (%)	Carbohydrate (%)
0%	19.59	6.35	6.07
10%	27.33	11.55	4.20
25%	35.07	16.70	3.02
50%	42.81	21.93	1.50

were used as the growth medium in this study. All materials were washed with chlorine-free water. Four combinations of "marine fish offal: tofu dreg", at 10:90; 50:50; 25:75, and 100% tofu dreg, were prepared as food for the Black Soldier Flies larvae and kept frozen for 24 hours before treatment. The proximate analysis was conducted on all feed (Table 1).

Fifty individuals of two-day old larvae were placed inside plastic cups (with three replicates for each treatment) and fed every 3 days with 150 mg (wet weight, 60% moisture content) of the food prepared according to the treatment. The larvae were initially placed onto the prepared and defrosted food inside the plastic cup. Larval weight and weight of remaining food were measured every 3 days until 40% of all larvae metamorphed into prepupae when feeding was also stopped (Tomberlin *et al.* 2002). All prepupae were removed daily from each container and weighed, then placed in plastic container and prepared for extraction.

All experiments were conducted at room temperature of 30 - 32 °C, relative humidity of 65 - 85%, and 12 hour photoperiod.

Sample Preparation for Fatty Acid Analysis

Extraction

All prepupae were cleaned by fresh water and dried at 60 °C for 12 hours. About 0.15 g of dried sample was ground then mixed with 3 mL solvent (2 part Chloroform and 1 part methanol). The mixture was then homogenized for 30 min, centrifuged at 5,400 rpm at 4 °C for 5 to 15 min and the supernatant was then collected. Homogenization and centrifugation processes were repeated 3 times. The collected supernatant was then evaporized to remove the organic solvent.

Esterification

The crude lipid sample was mixed with 4 mL NaOH 0.5 M with methanol solvent. About 0.024% Butylated Hydroxytoluene (BHT) was added, and then the solution was agitated for 8 hours at 55 °C.

Transesterification

Samples obtained after the esterification process were added with 300 mL methanol and 1 - 2.5% H_2SO_4 (v/v) in MeOH, and then agitated for 1 hour. The n-Hexane and NaCl (1 : 1) solution was added to the samples and then centrifugated for 10 min (4,000 g) at room temperature. Na_2SO_4 was then added in order to remove the excess water from the samples, and the supernatant was collected and kept in a separate container.

GC-FID Analysis for Omega 3 Content

The Omega 3 content analysis was conducted using Gas Chromatography-Flame Ionzation Detection (GC-FID). GC Column used was DB-5 30 m x 0.25 mm ID x 0.25 μm with N_2 and H_2 as the carrier gases. The applied injector temperature was 200 °C and the detector temperature was 300 °C. The oven temperature which started at 140 °C was increased to 270 °C, at a rate of 4 °C/min, and maintained for 7.5 min. The fatty acid was quantified with external standard method based on the formula:

$$Cs = \frac{As}{Aes} \times Ces$$

where:
Cs = Sample concentration
As = Sample area
Aes = External standard area
Ces = External standard concentration

Data Analysis

The final weight of harvested prepupae were compared across all diet composition using analysis of variance, with significance level set at $P < 0.05$, followed by the Tukey-Kramer HSD test when significant differences were detected. All analyses were done using SPSS version 22.0.

RESULTS AND DISCUSSION

The black soldier fly larvae were able to recycle a proportion of fish waste which consists of high protein and fat. The 10% of fish offal fed to the larvae has slightly improved the weight of harvested prepupae. However, the addition of more fish offal at 50% showed negative correlation to harvested weight, although not significant (Table 2).

The high mortality of larvae when fed with higher fish offal concentration was probably caused by: 1. lack of balance in the protein: carbohydrate combination of the feeding material. Both of these macromolecules are very important for growth, reproduction and survival of insects (Aguila *et al.* 2013). Proximate analysis showed the protein : carbohydrate level of 28.54 : 1 (Table 1). Studies showed that high protein content of the feed material produced high mortality rate in BSFL population (Cammack & Tomberlin 2017; Barragan-Fonseca *et al.* 2019; Danieli *et al.* 2019); 2. the production of juice through natural decomposition of the fish offal. This juice increased the moisture of feeding material, reduced the amount of available O_2 required for growth of insect larvae, and induced the molting process (Harrison *et al.* 2006; Klok & Harrison 2009; Harrison & Haddad 2011; Callier & Nijhout 2011).

Since BSFL were subjected to specific diet restrictions, the consumption of one diet component has altered the intake of other components. Since larval weight is highly associated with carbohydrate content (Le Gall & Behmer 2014; Barragan-Fonseca 2019) this probably explains the presence of heavier larvae at 25% fish offal.

Larvae fed with 25% and 50% fish offal required less development time to metamorph into the harvested prepupae (Table 1). The shorter development time of BSFL reared on high protein diet in the current study agrees with other results reported by Oonincx *et al.* (2015b) and Cammack & Tomberlin (2017). The development time of larvae in this study was even lower than those of other studies (Fatchurochim *et al.* 1989; Myers *et al.* 2008; Diener 2009; Nguyen *et al.* 2013; Oonincx *et al.* 2015a, b; St-Hilaire *et al.* 2007b; Cammack & Tomberlin 2017). This is probably related to the higher rearing temperature used in the current study. However, this result indicated the potential applicability of this method in tropical region like Indonesia.

The growth pattern showing weight gain was stopped at different times among the groups. Larvae at groups of 25% and 50% fish offal, stopped gaining weight at 16th day while those of groups 0% and 10% fish offal at 19th day (Fig. 1). The peak weights among groups were similar indicating that variations in the food composition did not affect the critical weight for prepupae development.

Table 2 Final weight and development time of harvested prepupae

Proportion of fish offal: tofu dreg* (%)	Fresh weight per 50 prepupae (g)	Development time (day)	Dry weight per 50 prepupae (g)
0	8.03 ± 0.09^{a}	20	3.00 ± 1.41^{a}
10	8.07 ± 0.02^{a}	20	3.10 ± 0.65^{a}
25	8.10 ± 0.12^{a}	19	3.74 ± 0.21^{a}
50	7.81 ± 1.26^{a}	19	2.90 ± 0.40^{a}

Note: * = Percent combination of fish offal: tofu dreg

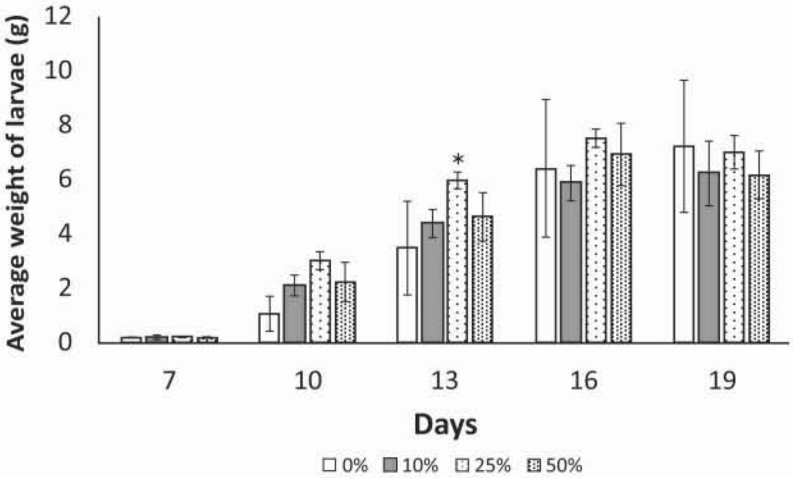

Figure 1 Changes in the weight of black soldier fly larvae fed with various combination of fish offal and tofu dreg
Notes: Data on 50% fish offal are not shown as more than 40% of larvae already metamorphosed into prepupae; * = significant at $P < 0.05$.

Most of the fatty acid content of BSFP biomass consisted of saturated fatty acid (Fig. 2). Similar results of highly saturated fatty acid content of black soldier fly prepupae were also obtained by other studies (St-Hilarie 2007b; Finke 2013; Ramos-Bueno *et al.* 2016; Surendra *et al.* 2016; Renna *et al.* 2017; Schiavone *et al.* 2017; Spranghers *et al.* 2017). The feeding material rich in carbohydrate but low in protein produced more crude fat (Barragan-Fonseca 2019) which is related to lipogenic activity and incorporation of lipid as body reserve (Nestel & Nemny-Lavy 2008).

The higher saturated fatty acid content in 10% fish offal group indicated the possibility of BSFP converting the unsaturated fatty acid into saturated fatty acid while higher fat content of the 50% fish offal group maybe responsible for the higher content of unsaturated fatty acid (Danieli *et al.* 2019).

Further analysis on each component of fatty acid showed high content of C12:0 (Lauric Acid) (Table 3). This conformed with other studies on the bioconversion of organic wastes by black soldier fly larvae (St-Hilarie 2007b; Sealey *et al.* 2011; Leong *et al.* 2015; Spranghers *et al.* 2017). The increase of lauric acid content associated with the decreasing amount of tofu dreg indicated the possible accumulation of lauric acid from fish offal as tropical fish tissues are rich in saturated fatty acid (Gopakumar & Nair 1972). The high content of this fatty acid might be related to the antibacterial function of insect larvae (Urbanek *et al.* 2012).

Richness in C12 : 0 found in BSF favor its inclusion in poultry feed since this fatty acid is better absorbed and metabolized than the long chain fatty acids (LCFA) for nutrition and for antibacterial activities (Skrivanova *et al.* 2006; Kim & Rhee 2016). However, higher saturated fatty acid content of the harvested prepupae could also increase the saturated fatty acid content of meat when it was applied as feed, a condition which is undesirable for modern consumers who prefer healthier meat and meat products (Schiavone *et al.* 2017).

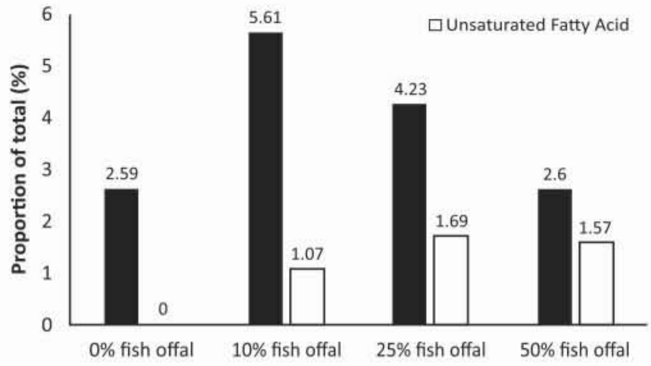

Figure 2 Proportion of saturatted and unsaturatted fatty acid of harvested prepupae (% dry matter basis)

Table 3 Fatty acid compostion of black soldier fly prepupe (% dry matter basis)

Lipid acid	Diet combination			
	0% fish offal	10% fish offal	25% fish offal	50% fish offal
C 10:0	0.04 ± 0.00	0.12 ± 0.06	0.10 ± 0.07	0.19 ± 0.21
C 12:0	1.05 ± 0.12	2.89 ± 1.12	3.09 ± 0.27	4.90 ± 4.16
C 14:0	0.12 ± 0.01	0.35 ± 0.14	0.28 ± 0.20	0.10 ± 0.10
C 16:1	0.09 ± 0.02	0.31 ± 0.14	0.24 ± 0.17	0.55 ± 0.49
C 16:0	0.93 ± 0.45	0.50 ± 0.22	0.21 ± 0.22	0.09 ± 0.12
C 18:0	0.19 ± 0.23	0.19 ± 0.09	0.46 ± 0.55	0.95 ± 0.91
C 18:3n-3; ALA	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.05
C 20:5	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.01
C 20:5n-3; EPA	0.02 ± 0.03	0.48 ± 0.22	0.93 ± 0.50	1.32 ± 1.13
C 22:6n-3; DHA	0.00 ± 0.00	0.39 ± 0.21	1.23 ± 0.29	1.22 ± 0.59
Total Omega-3	0.02	0.87	2.16	2.61

Omega-3 consists of three main lipid acids, ALA, EPA, and DHA. The amount of fish offal added to the diet was positively correlated with the amount of these molecules inside prepupae biomass. In general, the total amount of EPA and DHA of prepupae in this study was higher than similar study by St-Hillaire *et al.* (2007b) (Fig. 3). Low concentration of Omega 3 in prepupae might be related to the low Omega 3 content of tropical fish offal (Osman *et al.* 2001; Mohanty *et al.* 2016; Alhazzaa *et al.* 2018).

Combining fish wastes with other types of wastes could change the molecular properties of the biomass produced. Higher nutritional value of tofu dreg compared to cow manure which has much lower nutritional value might explain the difference of the results between this study and that of St-Hillaire *et al.* (2007b).

This study showed that fatty acids content of the black soldier fly prepupae could be increased and manipulated in order to produce desirable "healthy" fatty acids such as ALA, EPA, and DHA by feeding the larvae with more tropical marine fish waste (Barroso *et al.* 2017; Danielli *et al.* 2019). Although the application of this method as an Omega-3 source for human consumption would still require further studies, this method is already applicable in the production of a high-quality animal-grade foodstuff that is a suitable replacement for fish meal, fish oil, or vegetable oils in animal diets through the recycling of organic wastes. Furthermore, the application of Omega-3 rich feed material may change the fatty acid profile of the meat into a much healthier meat that is rich with unsaturated fatty acid (Saito *et al.* 1996; Li *et al.* 2016).

The fatty acid composition of BSF lipid highly depends on the rearing substrate (Makkar *et al.* 2014; Oonincx *et al.* 2015b; Leong *et al.* 2016). Therefore, further research is suggested to assess how much fatty acid can be improved through variation in substrate composition due to differences in fatty acid requirement for growth (Tocher 2010). When the fatty acid profile of BSF will be equal to the animal diets that contained fish meal/oil it will reduce the pressures on global marine fisheries and will also maintain environmental health (Tomberlin *et al.* 2015).

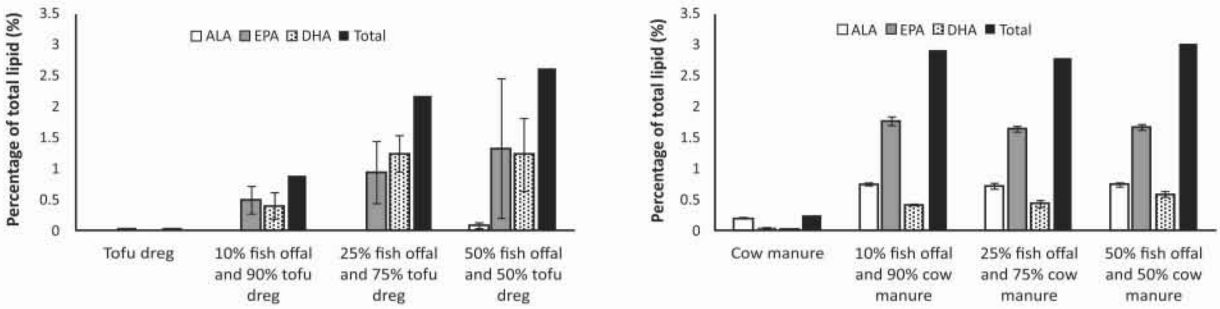

Figure 3 Percentage of a-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) on a total dry matter basis for prepupae feed diets containing different proportions of fish offal and tofu dreg (left) and results of Sheppard *et al.* (2007) (right)
Note: Error bars indicate the pooled SD.

CONCLUSION

The study results showed the possible applicability of a three-fronged solution (from basically a waste material), to three major human challenges, namely: 1. producing a high quality animal feed material from tropical marine fish wastes; 2. reducing the voluminous pile of organic wastes at the national level; and 3. producing a "healthier" meat for human consumption. The application of fish offal as feedstuff for black soldier fly larvae has also improved the total Omega 3 (EPA, DHA, and ALA) content of the harvested prepupae.

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