ACCEPTED MANUSCRIPT

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

Putra RE, Fatmalasari Y, Permana AD, Kinasih I, Rosmiati M

DOI: 10.11598/btb.……..

To appear in : BIOTROPIA Issue

Received date : 10 June 2018
Accepted date : 07 January 2020

This manuscript has been accepted for publication in BIOTROPIA journal. It is unedited, thus, it will undergo the final copyediting and proofreading process before being published in its final form.

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

Ramadhani Eka Putra[1*], Yenyen Fatmalasari[2], Agus Dana Permana[1], Ida Kinasih[3] and Mia Rosmiati[1]

[1]Agricultural Engineering Study Program, School of Life Sciences and Technology, Institut Teknologi Bandung, Bandung, Indonesia

[2]Biology Study Program, School of Life Sciences and Technology, Institut Teknologi Bandung, Bandung, Indonesia

[3]Departement of Biology, Universitas Islam Negeri Sunan Gunung Djati Bandung, Bandung, Indonesia

[*]Corresponding author, e-mail: ramadhani@sith.itb.ac.id

ABSTRACT

One of the materials with great potency for future nutrition source for animal feed is prepupae of black soldier fly larvae (BSFP) (*Hermetia illucens*) which is fed on organic wastes. This study was designed to observe the accumulation of specific substance (omega-3) of organic wastes (marine fish offal and tofu dregs) inside harvested biomass of BSFP. A total of 150 gram food consist of marine fish offal and tofu dreg with different proportion (10:90, 25:75, 50:50, and 100% tofu dregs) was fed to black soldier fly larvae (BSFL) for 21 days. At the end of feeding trial, all BSFL were harvested, weighed, and then analyzed for omega-3 fatty acids content. Fatty acid content was analyzed using Gas Cromatography with Flame Ionization Detector (GC-FID). Result of the experiment showed BSFL reared with 25% marine fish offal produced highest biomass (8.1 g / 50 larvae) with least development time (19 days). The total of omega-3 recorded from 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. This result showed the possibility of transferring specific nutrient from organic wastes biomass of BSFP which provides base knowledgement for further application in design of specific animal feed from BSFP.

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

INTRODUCTION

Most dominant wastes produced in Indonesia consisted of organic waste (Zulfikar & Chaerul 2010). Most of these ended as piles of wastes as collecting and waste management efforts only covered about 50-70% of wastes produced by population (Cointreau 1994; Rushbrook & Pugh 1999). Common methods applied to manage these wastes are costly sanitary landfill and composting which produce compost with low economic value, which make both methods less favorable in low income regions (Cointreau 1994).

Another alternative to manage organic waste is by conversion of organic wastes by saprophages through 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, Supriyatna & Putra 2017). Latest development showed the increasing trend of application of insect larvae as agent to decompost organic wastes as the process produces biomass rich in protein and lipid (Diener *et al.* 2009). Among them, 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 biomass which could be applied as sources of protein and lipid for high quality animal feed and potential source 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*. 2017, Renna *et al*. 2017, Spranghers *et al*. 2017; Kinasih *et al.* 2018).

Another benefit of applying *H. illucens* as organic wastes conversion agent, especially in tropical region like Indonesia, are (1) they are adapted to tropic and warm subtropical region (Tomberlin *et al.* 2002), (2) they are 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) they are able to reduce patogen like *Escherichia coli* and *Salmonella enterica* (Erickson *et al.* 2004), (4) they need 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). 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). Furthermore, like other insects, this digestive system also allowed them to accumulate certain organic molecules (St-Hilaire *et al*. 2007b).

All of these benefits could be harness to solve one of the main obstacles in food security of Indonesia, especially for protein, which is the continuous availability of affordable feed stock. Among organic wastes produced in large numbers in Indonesia, wastes with relatively unchange organic material properties during each production are tofu dreg and marine fish offal. A report showed about 731,501.5 tons tofu dreg was produced annually (Bisnis UKM 2009) while 25-30% of marine fish catch ended as wastes (KKP 2016). Tofu dreg is rich in carbohydrate and protein (Li *et al.* 2012, Li *et al.* 2013), while marine fish tissues rich in beneficial unsaturated fatty acids (Sahena *et al.* 2009) made them valuable organic wastes.

Studies showed that the nutritional content of BSFL biomass highly depends on quantity adn quality of the feeding material (Nguyen *et al.* 2015; Oonincx *et al.* 2015). Moreover, the fat content showed more variation (between 6.6 to 39.2%) than protein (between 37.0 to 62.7%) (Barragan-Fonseca *et al.* 2017) which suggest the strong effect of the larval dietary nutrient to protein and fat content. One of main purposes of BSFL production is as the 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). Based on the growing number of studies showed the possibility of modify the fatty acid profile of BSFL, there is possibility to improve the nutrient value (i.e. more healthy fats such as omega-3) of local livestock product through manipulation of feed leading to better value of the product as showed by study of Stoneham *et al.* (2018).

As 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). Study by St-Hilare *et al.* (2007b) showed that omega-3 content of BSFL could be enhanced by allowing them to feed on fish waste from rainbow trout processing plant. Unlike rainbow trout, most of marine tropical fish considered has 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 biomass. The main objective of this study to produced a BSFL with high omega-3 content at laboratory scale as a foundation for larger scale production of alternative source of Omega-3 produced through organic wastes conversion which applicable as livestock feed.

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 on the substance made of commercial chicken feed (60% moisture) and kept at constant temperature (28^{o}C, 70%RH) in a container (50 cm x 25 m x 10 cm) in Laboratory of Enviromental Toxicology, School of Life Sciences and Technology, Bandung, Indonesia.

Animal treatment

Growth medium applied in this study were marine fish offal originated from local fish market and tofu dreg produced as waste of local tofu industry of Sumedang, West Java. All materials were washed with chlorine free water. Four combinations of marine fish offal and tofu dreg, which were 10:90, 50:50, 25:75, and 100% tofu dreg, were prepared as food for larvae of

Black Soldier Flies and kept frozen for 24 hours before treatment. The proximate of the feed showed in Table 1.

Table 1 Proximate analysis of the feeding material

Proportion of fish offal	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

Two-day old larvae was used in this study. Fifty larvae were placed inside plastic cup (three replicates for each treatment) and fed with 150 mg prepared food according the treatment per 3 days (wet weight, 60% moisture content). The larvae were initially placed onto the prepared food, which was already defrosted in plastic cup. Larval weight and weight of remained food was measured every 3 days until 40% of all larvae metamorphed into prepupae when the addition of diet ceased (Tomberlin *et al*. 2002). All prepupae were removed daily from each container and weighed, then placed in plastic container for extraction process preparation.

All experiments were conducted in room temperature at 30-32^{o}C, relative humidity 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 gram of dried sample was grinded then mixed with 3 ml solvent (2 part Chloroform and 1 part methanol). The mixture was then homogenized with homogenizer for 30 min, centrifuged at 5400 rpm at 4^{o}C for 5 to 15 min. Supernatant was then collected. Homogenization and centrifugation process were repeated 3 times. Collected supernatant then evaporized with evaporator to remove organic solvent.

Esterification

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 shaked at 55^{o}C for 8 hours.

Transesterification

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

GC-FID analysis for Omega 3 content

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

$$Cs = \frac{As}{Aes} \text{ x } Ces$$

whereas

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 set at $p < 0.05$, followed by the Tukey-Kramer HSD test when significant value was detected. All analyses were done in SPSS version 22.0.

RESULTS AND DISCUSSION

The result confirmed that black soldier fly larvae were able to recycle a proportion of fish waste which consists of high protein and fat. Larvae fed as much as 10% of fish offal slightly improved the weight of harvested prepupae. On the other hand, the addition of fish offal about 50% and more showed negative correlation to harvested weight (Table 2), which led to significant mortality of larvae (preliminary study, result not showed). However, result of the present experiment showed the final weight was relatively similar among all food combinations (Table 2).

The high mortality of larvae when fed with high fish offal concentration was probably caused by: (1) lack of protein:carbohydrate balance of the feeding material. Both of these macromolecule are the most important nutrient for growth, reproduction and survival of insects (Aguila *et al*. 2013). Proximate analysis showed the protein:carbohydrate showed 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), and (2) juice produced through natural decomposition of fish offal. This juice increased the moisture of feeding material, reduced the amount of O_2 available required for growth of insect larvae, and induced molting process (Harrison *et al.* 2006, Klok & Harrison 2009, Harrison & Haddad 2011, Callier & Nijhout 2011).

On the other hand, since BSFL are restricted to the specific diet, the consumption of one component of diet will altered the intake of other component. Since larval weight highly associated with carbohydrate content (Le Gall & Behmer 2014; Barragan-Fonseca 2019) this may explained the heavier larvae of 25% fish offal.

Table 2 Final weight and development time of harvested prepupae

Proportion of fish offal	Fresh weight per 50 prepupae (g)	Development Time	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]

Larvae fed with 25% and 50% fish offal required less development time to metamorph into harvested prepupae (Table 2). The lower development time of BSFL reared on high protein diet found in this study agreed with other results reported by Oonincx *et al.* (2015b) and Cammack & Tomberlin (2017). The development time of larvae in this study was considered lower than 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) which might be related to the higher rearing temperature during this study. On the other hand, this result showed potency of applying this method in tropical region like Indonesia.

The growth pattern showed weight gain was stopped at different time among groups. Both groups of 25% and 50% fish offal stop the weight gain at 16th day while group 0% and 10% fish offal at 19th day (Fig. 1). The peak weight among groups was similar which indicated that differences in the food composition did not effect the critical weight for prepupae development.

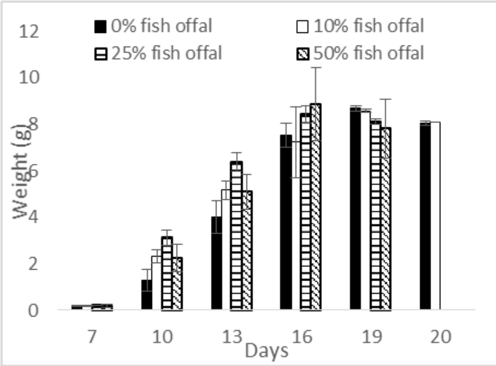

Figure 1 Changes in the wieght of black soldier fly larvae fed with various combination of fish offal and tofu dreg. Data of 50% fish offal not showed as more than 40% of larvae already metamorphosed into prepupae.

Most fatty acid content of BSFP biomass consisted of saturatted fatty acid (Fig. 2). This result agreed with other research showed high saturated fatty acid content of black soldier fly prepupae (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). On the other hand, feeding material rich in carbohydrate but low in protein produced more crude fat which agreed with Barragan-Fonseca (2019) which may related to lipogenic activity and incorporation of lipid as body reserve (Nestel & Nemny-Lavy 2008).

Higher saturated fatty acid in 10% fish offal group may also related to possibility BSFP to convert unsaturated fatty acid into saturated fatty acid while higher fat content of 50% fish offal group may responsible for higher content of unsaturated fatty acid (Danieli *et al.* 2019).

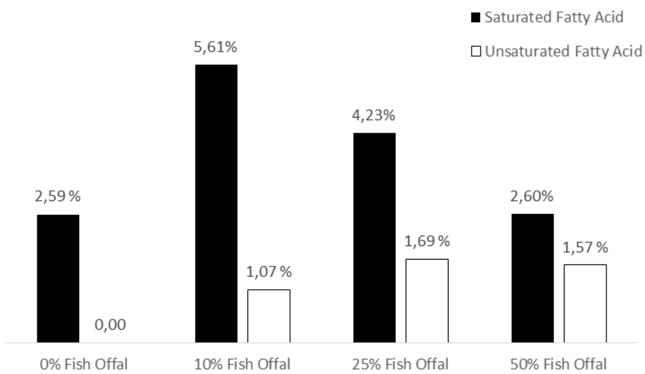

Figure 2 Proportion of saturatted and unsaturatted fatty acid of harvested prepupae (% of dry matter basis)

Further analysis on each component of fatty acid showed high content of C12:0 (Lauric Acid) (Table 3) which agreed with other studies on 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 with the decreasing amount of tofu dreg indicated the possibility of accumulation from fish offal as tropical fish tissues rich in saturated fatty acid (Gopakumar & Nair 1972). The high content of this fatty acid might be related to its function as antibacteria for insect larvae (Urbanek *et al*. 2012)

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

Table 3 Fatty acid compostion of black soldier fly prepupe (% of 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 is consisted of three main lipid acids, ALA, EPA, and DHA. The results of this study showed positive correlation between the amount of fish offal added to diet and 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) while the amount of ALA was much smaller (Fig. 3). Low ALA concentration in prepupae produced in this study might be related to low ALA content of tropical fish offal (Osman *et al.* 2001, Mohanty *et al*. 2016).

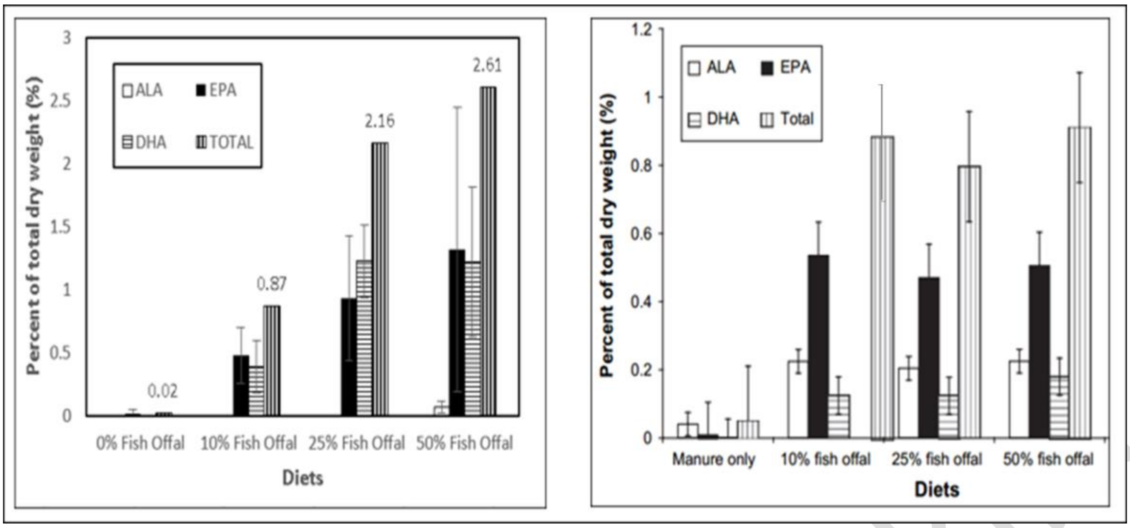

Figure 3 Percentage of a-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) on a total dry matter basis for prepupae fed diets containing different proportions of fish offal (left) and result of Sheppard *et al.* (2007) (right). Error bars indicate the pooled SE.

Combining fish waste with different types of waste could change the properties of molecules of biomass produced. Higher nutrient nutritional value of tofu dreg compared to cow manure which has much lower nutritional value may explain the difference of the results between this study and similar study of St-Hillaire *et al.* (2007b).

This study showed fatty acids content of black soldier fly prepupae can be increased and manipulated in order to produce desirable “healthy” fatty acids such as ALA, EPA, and DHA by feeding the larvae with tropical marine fish waste which is agreed with Barroso *et al.* (2017) and Danielli *et al.* (2019). Although the application of this method as source of omega 3 in human would require further studies, this method could be applicable to produce a high-quality animal-grade foodstuff that is a suitable replacement for fish meal, fish oil, or vegetables oil in animal diets through recycle of organic wastes. Furthermore, the application of feed material rich in omega-3 may change the fatty acid profie of the meat into much healthier meat 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 efforts should be carried out in order to assess how much the fatty acid profile of lipidt can be improved through substrate composition due to the differences in fatty acid requirement for growth (Tocher 2010). When the fatty acid profile of BSF was equal to the animal diets that contained fish meal/oil it will reduce the pressures on global marine fisheries and maintain environment health (Tomberlin *et al.* 2015).

CONCLUSION

The application of fish offal as feedstuff for black soldier fly larvae improved the total omega 3 (EPA, DHA, and ALA) content of harvested prepupae. This study showed the possibility to produce high quality animal feed material from tropical marine fish waste.

ACKNOWLEDGEMENTS

This study was partly and equally supported by research grant of Indofood Riset Nugraha and Riset Kompetensi Kemenristek DIKTI.

REFERENCES

Aguila JR, Hoshizaki DK, Gibbs AG (2013) Contribution of larval nutrition to adult reproduction in Drosophila melanogaster. J. Exp. Biol. 216:399-406

Barragan-Fonseca KB, Gort G, Dicke M, van Loon JJA. 2019. Effects of dietary protein and carbohydrate on life-history traits and body protein and fat contents of the black soldier fly *Hermetia illucens*. Physiol. Entomol 44(2):148-159.

Barnard DR, Harms RH, Sloan DR. 1998. Biodegradation of poultry manure by house fly (Diptera: Muscidae). Environ. Entomol. 27:600-5.

Barroso FG, Sánchez-Muros M-J, Segura M, Morote E, Torres A, Ramos R, Guil J-L. 2017. Insects as food: Enrichment of larvae of *Hermetia illucens* with omega 3 fatty acids by means of dietary modifications. J Food Compos Anal 62:8-13.

Barry T. 2004. Evaluation of the economic, social, and biological feasibility of bioconverting food wastes with the black soldier fly (*Hermetia illucens*). *Doctor of Philosophy (Environmental Science) Dissertation*. Texas : University of North Texas.

Beard RL, Sands DC. 1973. Factors affecting degradation of poultry manure by flies. Environ. Entomol. 2:801-6.

Bisnis UKM. (2009). Sedapnya Bisnis Kecap Ampas Tahu. http://bisnisukm.com/sedapnya-kecap-dari-ampas-tahu.html. Accessed: 30 Juli 2017.

Callier V, Nijhout HF. 2011. Control of body size by oxygen supply reveals size-dependent and size-independent mechanisms of molting and metamorphosis. PNAS 108(35):14664-9.

Cammack JA, Tomberlin JK. 2017. The impact of diet protein and carbohydrate on select life-history traits of the black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae). Insects 8:56.

Cointreau-Levine, S. (1994). *Private sector participation in municipal solid waste services in developing countries. Vol. 1. The formal sector. Urban Management Programme Discussion Paper No. 13*. World Bank. Washington, United States.

Danieli PP, Lussiana C, Gasco L, Amici A, Ronchi B. 2019. The Effects of Diet Formulation on the Yield, Proximate Composition, and Fatty Acid Profile of the Black Soldier Fly (*Hermetia illucens* L.) Prepupae Intended for Animal Feed. Animals 9(4):178.

Diener S, Zurbrügg C, Tockner K. 2009. Conversion of Organic Material by Black Soldier Fly Larvae: estabilishing optimal feeding rates. Waste Manage Res. 27:603-10.

El Boushy A 1991. House fly pupae as poultry manure converters for animal feed: a review. Bioresour Technol. 28:45-9.

Erickson MC, Islam M, Sheppard C, Liao J, Doyle MP. 2004. Reduction of *Esscherichia coli* O157:H7 and *Salmonella enterica* Serovar Enteritidis in chicken manure by larvae of black soldier fly. J. Food Prot. 67: 685-90.

Fatchurochim S, Geden CJ, Axtell RC. 1989. Filth fly (Diptera) oviposition and larval development in poultry manure of various moisture levels. J. Entomol. Sci.. 24:224-31.

Finke MD. 2013. Complete nutrient content of four species of feeder insects. Zoo. Biol. 32: 27-36.

Furman D, Young R, Catts EP. 1959. *Hermetia illucens* (Linaeus) as a factor in the natural control of *Musca domestica* Linnaeus. J. Econ. Entomol. 52:917-21.

Gopakumar K, Nair MR. 1972. Fatty acid composition of eight species of Indian marine fish. J. Sci. Food Agric. 23:493–6.

Harrison J, Frazier MR, Henry JR, Kaiser A, Klok CJ, Rascon B. 2006. Responses of terrestrial insects to hypoxia or hyperoxia. Respir Physiol. Neurobiol. 154:4-17.

Harrison JF, Haddad GG. 2011. Effects of oxygen on growth and size: Synthesis of molecular, organismal, and evolutionary studies with *Drosophila melanogaster*. Annu. Rev. Physiol. 73:95-113.

Kementrian dan Kelautan Republik Indonesia (KKP). (2016). Konsumsi Ikan Naik dalam 5 Tahun Terakhir. http://kkp.go.id/index.php/berita/konsumsi-ikan-naik-dalam-5-tahun-terakhir/. Accessed: 30 Maret 2017

Kim SA, Rhee MS. 2016. Highly enhanced bactericidal effects of medium chain fatty acids (caprylic, capric, and lauric acid) combined with edible plant essential oils (carvacrol, eugenol, b-resorcylic acid, trans-cinnamaldehyde, thymol, and vanillin) against *Escherichia coli* O157:H7. Food Control 60:447–54.

Kim W, Bae S, Lee S, Choi Y, Han S, Koh Y. 2011. Biochemical characterization of digestive enzymes in the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). J. Asia Pac. Entomol. 14(1):11-4.

Klok CJ, Harrison JF. 2009. Atmospheric hypoxia limits selection for large body size in insects. PLoS ONE 4:e3876.

Le Gall, M. & Behmer, S.T. (2014) Effects of protein and carbohydrate on an insect herbivore: the vista from a fitness landscape. Integrative and Comparative Biology, 54, 942–954.

Leclercq, M. 1997. A propos de *Hermetia illucens* (Linnaeus, 1758) “soldier fly” (Diptera Stratiomyidae: Hermetiinae). Bulletin et Annales de la Societe Royale Belge d’Entemologie 133:275-282.

Leiber, F., Gelencsér, T., Stamer, A., Amsler, Z., Wohlfahrt, J., Früh, B. and Maurer, V., 2017. Insect and legume-based protein sources to replace soybean cake in an organic broiler diet: effects on growth performance and physical meat quality. Renew. Agr. Food. Syst. 32:21-27

Leong, S.Y., Kutty, S.R.M., Tan, C.K., Tey, L.H. 2015. Comparative study on the effect of organic waste on lauric acid produced by *Hermetia illucens* larvae via bioconversion. Journal of Engineering Science and Technology, Special Issue on ACEE 2015 Conference, 52-63.

Leong SY, Kutty SRM, Malakahmad A, Tan CK. 2016. Feasibility study of biodiesel production using lipids of *Hermetia illucens* larva fed with organic waste. Waste Manage 47:84–90

Li B, Lu F, Nan H, Liu Y. 2012. Isolation and structural characterisation of okara polysaccharides. Molecules 17:753–61

Li S, Ji H, Zhang B, Tian J, Zhou J, Yu H. 2016. Influence of black soldier fly (*Hermetia illucens*) larvae oil on growth performance, body composition, tissue fatty acid composition and lipid deposition in juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquaculture 465:43-52.

Li S, Zhu D, Li K, Yang Y, Lei Z, Zhang Z. 2013. Soybean Curd Residue: Composition, Utilization, and Related Limiting Factors, ISRN Industrial Engineering 2013: Article ID 423590

Li Q, Zheng L, Qiu N, Cai H, Tomberlin JK, Yu Z. 2011. Bioconversion of dairy manure by black soldier fly (Diptera: Stratiomyidae) for biodiesel and sugar production. Waste Manage 31:1316-20.

Makkar HPS, Tran G, Heuze V, Ankers P. 2014. State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Technol. 197:1-33.

Manurung R, Supriatna A, Esyanthi RE, Putra RE. 2016. Bioconversion of Rice straw waste by blacksoldier fly larvae (*Hermetia illucens* L.): Optimal feed rate for biomass production. J. Entomol. Zool. Stud. 4(4):1036-41.

Mohanty BP, Ganguly S, Mahanty A, Snkar TV, Anandan R, Chakraborty K, Paul BN, Sarma D, Dayal JS, Venkateshwarlu G, Mathew S, Asha KK, Karunakaran D, Mitra T, Chandra S, Shahi N, Das P, Akhtar MdS, Vijayagopal P, Sridhar N. 2016. DHA and EPA content and fatty acid profile of 39 food fishes from India. Biomed Res. Int. 2016: Artikel ID 4027437.

Muin H, Taufek NM, Kamarudin MS, Razak SA. 2017. Growth performance, feed utilization and body composition of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) fed with different levels of black soldier fly, *Hermetia illucens* (Linnaeus, 1758) maggot meal diet. Iran J. Fish Sci. 16(2):567-77.

Myers H, Tomberlin JK, Lambert B, Kattes D. 2008. Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure. Environ. Entomol. 37:11-5.

Ndegwa PM, Thompson SA. 2001. Integrating composting and vermicomposting in the treatment and bioconversion of biosolids. Bioresour Technol. 76:107-12.

Nestel D, Nemny-Lavy E. 2008. Nutrient balance in medfly, Ceratitis capitata, larval diets affect the ability of the developing insect to incorporate lipid and protein reserves. Entomologia Experimentalis et Applicata, 126, 53–60

Newby R. 1997. *Use soldier fly larvae in organic waste management*. Proceedings of the ‘Compost 97’ Conference, 14-15 July 1997, Griffith University, Brisbane Hilton, Bribane. Griffith University, Brisbane , Australia.

Newton GL, Booram CV, Barker RW, Hale OM. 1977. Dried *Hermetia illucens* larvae meal as a supplement for swine. J. Anim. Sci. 44:395-400.

Newton GL, Sheppard DC, Watson DW, Burtle GJ, Dove CR. 2005a. *Using the black soldier fly, Hermetia illucens, as a value added tool for management of swine manure*. Animal and Poultry Waste Management Center, North Carolina State University, Raleigh, NC. pp. 17.

Newton GL, Sheppard DC, Watson DW, Burtle GJ, Dove CR, Tomberlin JK, Thelen EE. 2005b. *The black soldier fly, Hermetia illucens, as a manure management/resource recovery tool*.

Symposium on the state of the science of Animal Manure and Waste Management, San Antonio, Texas, USA.

Nguyen TTX, Tomberlin JK, Vanlaerhoven S. 2013. Influence of resources on *Hermetia illucens* (Diptera: Stratiomyidae) larval development. J. Med. Entomol. 50:898–906.

Oonincx DGAB, van Huis A, van Loon JJA. 2015a. Nutrient utilisation by black soldier flies fed with chicken, pig, or cow manure. J. Insects Food Feed 1:131–9.

Oonincx DGAB, van Broekhoven S, van Huis A, van Loon JJ. 2015b. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. PLoS ONE 10:e0144601

Osman H, Suriah AR, Law EC. 2001. Fatty acid composition and cholesterol content of selected marine fish in Malaysian waters. Food Chem. 73:55–60.

Rachmawati, Buchori D, Hidayat P, Hem S, Famhi MR. 2010. Development and nutrien content of larva *Hermetia illucens* (Linnaeus) (Diptera: Stratiomyidae) on palm kernel meal (Perkembangan dan Kandungan Nutrisi Larva *Hermetia illucens* (Linneus) (Diptera: Stratiomyidae) pada Bungkil Kelapa Sawit). Jurnal Entomologi Indonesia 7:28-41.

Ramos-Bueno RP, Gonzalez-Fernandez MJ, Sanchez-Muros Lozano MJ, Barroso FG, Guil-Guerrero JL. 2016. Fatty acid profiles and cholesterol content of seven insect species assessed by several extraction systems. Eur. Food Res. Technol. 242:1471–7.

Ramos-Elorduy J, Gonzalez EA, Hernandez AR, Pino JM. 2002. Use of *Tenebrio molitor* (Coleoptera: Tenebrionidae) to recycle organic wastes and as feed for broiler chickens. J. Econ. Entomol. 95:214-20.

Renna M, Schiavone A, Gai F, Dabbou S, Lussiana C, Malfatto V, Prearo M, Capucchio MT, Biasato I, Biasibetti E, De Marco M, Brugiapaglia A, Zoccarato I, Gasco L. 2017. Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens* L.) larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss* Walbaum) diets. J. Anim. Sci. Biotechnol. 8:57.

Rushbrook PE, Pugh MP. 1999. *Solid Waste Landfills in Middle and Lower Income Countries: a technical guide to planning, design and operation*. *World Bank Technical Paper No. 426*. World Bank, Washington DC, USA.

Sahena F, Zaidul ISM, Jinap S, Saari N, Jahurul HA, Abbas KA, Norulaini NA. 2009. PUFAs in Fish: Extraction, fractionation, importance in health. Compr. Rev. Food Sci. Food Saf 8:59–74.

Saito H, Ishihara K, Murase T. 1996. Effect of prey fish lipids on the docosahexaenoic acid content of total fatty acids in the lipid of *Thunnus albacares* yellowfin tuna. Biosci. Biotechnol. Biochem. 60(6):962-5.

Schiavone A, Cullere M, De Marco M, Meneguz M, Biasato I, Bergagna S, Dezzutto D, Gai F, Dabbou S, Gasco L, Zotte AD. 2017. Partial or total replacement of soybean oil by black soldier fly larvae (*Hermetia illucens* L.) fat in broiler diets: effect on growth performances, feed-choice, blood traits, carcass characteristics and meat quality. Ita J. Anim. Sci. 16(1):93-100.

Sealey WM, Gaylord TG, Barrows FT, Tomberlin JK, McGuire MA, Ross C, St-Hilaire S. 2011. Sensory analysis of rainbow trout, *Oncorhynchus mykiss* fed enriched black soldier fly prepupae, *Hermetia illucens*. J. World Aquacult. Soc. 42(1):34-45.

Skrivanova E, Marounek M, Benda V, Brezina P. 2006. Susceptibility of *Escherichia coli*, *Salmonella* sp. and *Clostridium perfringens* to organic acids and monolaurin. Vet. Med-Czech 51(3):81–8.

Spranghers T, Ottoboni M, Klootwijk C, Ovyn A, Deboosere S, De Meulenaer B, Michiels J, Eeckhout M, De Clercq P, De Smet S. 2017. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. J. Sci Food Agric. 97:2594–600.

Ssepuuya G, Namulawa V, Mbabazi D, Mugerwa S, Fuuna P, Nampijja Z, Ekesi S, Fiaboe KKM, Nakimbugwe D. 2017. Use of insects for fish and poultry compound feed in sub-Saharan Africa - a systematic review. J. Insect Food Feed 3(4):289-302.

Stadtlander T, Stamer A, Buser A, Wohlfahrt J, Leiber F, Sandrock C. 2017. Hermetia illucens meal as fish meal replacement for rainbow trout on farm. J. Insect Food Feed 3(3):165-175.

Stanley-Samuelson DW, Dadd RH. 1983. Long-chain polyunsaturated fatty acids: patterns of occurrence in insects. Insect Biochem. 13:549–58.

St-Hilaire S, Sheppard C, Tomberlin JK, Irving S, Newton L, Mcguire MA, Mosley EE, Hardy RW, Sealey W. 2007a. Fly prepupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*. J. World Aquacult. Soc. 38(1):59-67.

St-Hilaire S, Cranfill K, McGuire MA, Mosley EE, Tomberlin JK, Newton L, Sealey W, Sheppard C, Irving S. 2007b. Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. J. World Aquacult. Soc. 38:309–13.

Stoneham TR, Kuhn DD, Taylor DP, Neilson AP, Smith SA, Gatlin DM, Chu HSS, O'Keefe SF. 2018. Production of omega-3 enriched tilapia through the dietary use of algae meal or fish oil: Improved nutrient value of fillet and offal. PLoS One 13(4):e0194241.

Supriyatna A, Manurung R, Esyanthi RE, Putra RE. 2016. Growth of black soldier larvae fed on cassava peel wastes, An agriculture waste. J. Entomol. Zool. Stud. 4(6):161-5.

Supriyatna A, Putra, RE. 2017. Estimasi Pertumbuhan Larva Lalat Black Soldier (*Hermetia illucens*) dan Penggunaan Pakan Jerami Padi yang Difermentasi dengan Jamur *P. chrysosporium.* Jurnal Biodjati 2(2):159-66.

Surendra KC, Olivier R, Tomberlin JK, Jha R, Khanal SK. 2016. Bioconversion of organic wastes into biodiesel and animal feed via insect farming. Renew Energ. 98: 197–202.

Thompson SN. 1973. A review and comparative characterization of the fatty acid composition of seven insect orders. Comp. Biochem. Physiol. 45B:467–82

Tocher DR. 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. Aquacult. Res. 41:717–32.

Tomberlin JK, Sheppard DC, Joyce JA. 2002. A comparison of selected life history traits of the black soldier fly (Diptera : Stratiomyidae) when reared on three diets. Ann. Entomol. Soc. Am. 95:379-87.

Tomberlin JK, van Huis A, Benbow ME, Jordan H, Astuti DA, Azzollini D, Banks I, Bava V, Borgemeister C, Cammack JA, Chapkin RS, Čičková H, Crippen TL, Day A, Dicke M, Drew DJW, Emhart C, Epstein M, Finke M, Fischer CH, Gatlin D, Grabowski NTh, He C, Heckman L, Hubert A, Jacobs J, Josephs J, Khanal SK, Kleinfinger JF, Klein G, Leach C, Liu Y, Newton GL, Olivier R, Pechal JL, Picard CJ, Rojo S, Roncarati A, Sheppard C, Tarone AM, Verstappen B, Vickerson A, Yang H, Yen AL, Yu Z, Zhang J, Zheng L. 2015.

Protecting the environment through insect farming as a means to produce protein for use as livestock, poultry, and aquaculture feed. J. Insects Food Feed, 1;307–9.

Urbanek A, Szadziewski R, Stepnowski P, Boros-Majewska J, Gabriel I, Dawgul M, Kamysz W, Sosnowska D, Golebiowski M. 2012. Composition and antimicrobial activity of fatty acids detected in the hygroscopic secretion collected from the secretory setae of larvae of the biting midge *Forcipomyia nigra* (Diptera: Ceratopogonidae). J. Insect Physiol. 58:1265-76.

van Huis A, van Itterbeeck J, Klunder H, Mertens E, Halloran A, Muir G, Vantomme P. 2013. Edible insects: future prospects for food and feed security. FAO Forestry Paper 171. Rome: FAO (Food and Agriculture Organization of the United Nations). URL: www.fao.org/docrep/018/i3253e/i3253e.pdf. Accessed: 30 Juli 2017.

Webster, C.D., Rawles, S.D., Koch, J.F., Thompson, J.R., Kobayashi, Y., Gannam, A.L., Twibell, R.G. and Hyde, N.M., 2015. Bio-Ag reutilization of distiller's dried grains with solubles (DDGS) as a substrate for black soldier fly larvae, Hermetia illucens, along with poultry by-product meal and soybean meal, as total replacement of fishmeal in diets for Nile tilapia, Oreochromis niloticus. Aquaculture Nutrition 22: 976-988.

Zulfikar, Chaerul, M. 2010. Evaluation of Waste Management at Kecamatan Sukasari, Bandung (Evaluasi Sistem Pengelolaan Sampah di Kecamatan Sukasari, Bandung). *Thesis*. Bandung : Faculty of Civil and Environmental Engineering, Institut Teknologi Bandung.