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Factors affecting the bioconversion of Philippine tung seed by black soldier fly larvae for the production of protein and oil-rich biomass

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ABSTRACT

A systematic study on the use of Philippine tung (*Reutealis trisperma)* seed as a substrate for the cultivation of black soldier fly larvae (*Hermetia illucens*) was performed. The characteristics of *Reutealis trisperma* seed from two different locations: West Java and Papua, were determined. The seed has a relatively high oil (37.6–39.2%, dry weight) and protein content (14.9–28.2%, dry weight). The effect of cake content in the substrate (0–20%, wet weight), moisture content in the substrate (50–70%, wet weight), feeding rate (50–100 mg/larva/d), lighting condition (dark-light) and substrate depth in a rearing container (4–10 cm) was performed. An optimum pre-pupal biomass productivity of 123.4 g/m^2/d was obtained (20%, wet weight of cake content in the substrate, 60%, wet weight of moisture content in the substrate, 100 mg/larva/d, dark, 6 cm substrate depth). The protein and oil content of the biomass were also determined to evaluate the effect of *Reutealis trisperma* seed as a substrate for the cultivation of black soldier fly larvae to produce protein and oil-rich biomass. The oil content in the biomass was also extracted and the fatty acid composition was identified. The prepupal biomass has a relatively high amount of protein (45%, dry weight) and oil content (26.6%, dry weight) and is suitable for cattle feed application.

Introduction

The Philippine tung tree (*Reutealis trisperma*) is an evergreen tree that is widely scattered in forests at low and medium altitudes in Philippines. This tree is also cultivated in other countries such as Indonesia, China and India for its timber and medicinal use. The tree produces seed which has not yet being valorized. The productivity of Philippine tung (trisperma) seeds is reported to be in the range of 3.8–8.7 ton/ha/yr (Kumar et al., 2015; Agricultural Office of West Java). The identification of added values from trisperma seeds, using a biorefinery concept is highly relevant to increase the overall value of the trisperma tree.

Trisperma seeds consist of 65% kernels by weight (wt%) on a dry basis (d.b) and 35 wt%, d.b. shells. The kernels have an oil content in the range of 58–61 wt% d.b. which corresponds to 38–40 wt%, d.b. oil content on a seed basis (Abduh et al., 2018). The oil, also known as trisperma oil can be isolated from the kernel and may be a valuable source for biodiesel production and may find applications as ingredients in making soap and paints (Holilah et al., 2015; Kumar et al., 2015). The cake obtained after the isolation of oil from the kernels may be utilized as cattle feed because it contains a considerable amount of protein (Manurung et al., 2016). The remaining biomass, particularly the shell, is normally discarded as waste.

The shell containing a relatively high amount of lignocellulose may be mixed with the cake as a substrate for the cultivation of black soldier fly larvae (BSFL) (*Hermetia illucens* L.) to produce higher value added products such as protein and oil-rich biomass. BSFL is a non-pest fly that has a relatively high protein and fat content which lies in the range of 29–55 wt% and 19–39 wt%, respectively. Diener et al. (2009) demonstrated that the protein content of BSFL varied from 28 to 43 wt% when the larvae were fed with chicken feed which depends on the feeding rate. In another study by Abduh et al. (2017), BSFL was fed with rubber seeds and the protein content varied from 29 to 55 wt% whereas the oil content varied from 19 to 28 wt% which depends on whether the rubber seeds were pre-treated with consortium of microbes or not. St-Hillaire reported that BSFL fed with fish offal had on average 30 wt% oil, which was 43 wt% more than the BSFL fed with cow manure only. Another study by Zheng et al. (2011) demonstrated that the oil content of the BSFL reached up to 39 wt% when the BSFL was fed with restaurant solid waste. As such highlights that the protein and oil

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content of the BSFL highly depends on the substrate consumed by the larvae.

Studies on the conversion of organic material by BSFL to produce insect biomass have been reported in the literature (Diener et al., 2009; Zheng et al., 2012; Li et al., 2011). However systematic studies on factors affecting bioconversion of trisperma seeds for producing protein and oil-rich biomass are not yet available in open literature. Hence, this study aims to determine the factors affecting bioconversion of trisperma seed by BSFL, particularly the cake content in the substrate, moisture content in the substrate, substrate depth in a rearing container, and feeding rate, for producing insect biomass with a high level of protein and oil. The protein and oil content of the biomass were also determined to evaluate the potential of the produced insect biomass to be used as cattle feed.

Materials and methods

Hydraulic pressing of trisperma seeds

Ruetealis trisperma seeds from West Java were first dehulled to separate kernel from the shell. Trisperma oil was isolated from the kernel using a locally constructed laboratory scale hydraulic press (ITB, Bandung). Approximately 500 g of sample was placed in the pressing chamber and pressed at room temperature (27 °C) for 4–5 h (Abduh et al., 2017). The pressed oil was analyzed with GC–MS to determine the fatty acid composition and the pressed cake was used as a substrate for the cultivation of BSFL.

Soxhlet extraction of trisperma oil and BSFL oil

Ruetealis trisperma seeds from Papua were first dehulled to separate the kernels from the shells. Trisperma oil within the kernels was extracted using a Soxhlet extraction procedure. The kernel was dried at dried at 103 °C and ground using a coffee grinder. Approximately 7 g of sample was transferred into a Soxhlet timble and extracted with n-hexane (99 vol%, Bratachem, Bandung) for at least 5 h. The solvent was evaporated using a rotary evaporator (atmospheric pressure, 69 °C) and sample was subsequently dried at 103 °C until constant weight was achieved (Abduh et al., 2016).

The oil within the BSFL sample was also extracted using similar procedures. The oil content in the sample is reported as gram oil per gram sample on a dry basis. The extracted oil was analyzed with GC–MS to determine the fatty acid composition. The analyses were performed in triplicate and reported as an average value.

Cultivation of BSFL with the remaining biomass from trisperma seeds

Black soldier fly eggs were obtained from a local farmer at Sumedang, Indonesia. The eggs were initially hatched and later reared on chicken meal (60 wt% moisture) for 7 d. Twenty 7 d old BSFL were placed inside a rearing container and fed with the remaining biomass obtained after the isolation of oil from the trisperma seeds (Manurung et al., 2016). Due to limited availability of trisperma seeds from Papua, only trisperma seeds from West Java were used as a substrate for the cultivation of BSFL. The pressed cake was mixed with the shell (cake content: 0, 10, 20 wt%) before ground and sieved (12 mesh). The mixed biomass was added with water (moisture content: 50, 60, 70, wt%) and used as a substrate for the cultivation of BSFL. The feeding rate was set at 50, 75 and 100 mg/larvae/d. The depth of substrate provided to BSFL in the rearing container was also varied from 4, 6, 8, and 10 cm. Two types of rearing container were used in this study: i) a cylindrical container (diameter: 7 cm, substrate depth: 10 cm) ii) a rectangular container with (length: 26 cm, width: 16 cm, substrate depth: 4, 6, 8 and 10 cm). The rearing containers were subjected to different lighting conditions: dark and light. A dark condition implies that the rearing containers were covered by a black plastic cover (approximately 0 lx), ii) a light condition implies that rearing containers were covered by a transparent plastic cover (approximately 80–90 lx).

The larvae were weighed every 3 d and moved into a new container and fed with a fresh substrate. The weight of residues in the container that comprises of excretory products and unconsumed feed was recorded before and after drying at 103 °C. Data measurement was carried out until most of the larvae (at least 50% of the total population) in the rearing container had developed into prepupae (approximately 12–13 d). The prepupae were inactivated by drying at 103 °C until constant weight was achieved. All of the analyses were performed in triplicate and expressed as mean ± standard deviation. Differences were tested with one-way analysis of variance (ANNOVA) using MINITAB 17.

Data analysis

The efficiency of BSFL in converting the remaining biomass of *Ruetealis trisperma* seed into protein-rich biomass was accessed by calculating the assimilation efficiency (AE), efficiency of conversion of digested-feed (ECD) and waste reduction index (WRI) as suggested by Scriber and Slansky (1981) and Diener et al. (2009). The equations and description to calculate AE, ECD and WRI are described by Manurung et al. (2016).

Analytical methods

The lignocellulose content of the samples was determined based on the procedures by Manurung et al. (2016). The protein content of the sample was analyzed by Kjedahl method (SNI-01-2891-1992). The analysis was carried out at the Analytical Laboratory, University of Padjajaran, Jatinangor. The fatty acid composition of trisperma and BSFL oil was determined by gas chromatography-mass spectrometry (GC–MS) at the Chemical Laboratory, University of Education of Indonesia, Bandung. The analyses were performed in triplicate and reported as an average value.

Results and discussion

Characteristic of trisperma seeds

Trisperma seeds investigated in this study were obtained from two different locations: West Java and Papua, Indonesia. The initial moisture content of the seeds from West Java and Papua upon receipt was approximately 20.6 wt% and 26.8 wt%, respectively as shown in Table 1. The seeds from West Java consist of approximately 35.1 wt%, d.b. shells and 64.9 wt%, d.b. kernels whereas the seeds from Papua consist of approximately 32.7 wt%, d.b. shells and 67.3 wt%, d.b.

Table 1
Weight fraction, oil content, protein content and moisture content of trisperma seed, kernel and shell from West Java and Papua.

Property	West Java	Papua
Weight fraction, wt% d.b.		
Seed	100	100
Kernel	35.1	32.7
Shell	64.9	67.3
Oil content, wt% d.b.		
Seed	39.2	37.6
Kernel	60.3	56.4
Shell	0	0
Protein content, wt%, d.b.		
Seed	14.9	28.2
Kernel	32.7	67.8
Shell	5.2	9.0
Moisture content, % d.b.		
Seed	20.6	26.8
Kernel	22.0	–
Shell	16.8	–

Table 2
Fatty acid composition of trisperma oil West Java, Papua and other locations.

Fatty acid	West Java	Papua	East Java[a]	India[b]
Saturated fatty acid				
Palmitic acid (16:0)	15.2	9.2	22.9	13.1
Stearic acid (18:0)	6.6	2.3	22.0	5.7
Total saturated fatty acid	21.8	11.5	44.9	18.8
Unsaturated fatty acid				
Palmitoleic acid (16:1)	–	–	0.3	
Oleic acid (18:1)	18.4	51.2	30.2	15.4
Linoleic acid (18:2)	23.1	37.3	13.6	17.4
Linolenic acid (18:3)	–	–	1.8	–
α-eleostearic acid (18:3)	36.8	–	–	–
Arachidic acid (20:0)	–	–	1.1	–
Nervonic acid (24:1)	–	–	–	44.1
Docosahexaenoic acid (22:6)	–	–	–	2.4
Total unsaturated fatty acid	78.3	88.5	47.0	79.3

[a] Holilah et al. (2015).
[b] Kumar et al. (2015).

kernels. The oil in the seeds from West Java (39.2 wt%, d.b.) is slightly higher than the sample from Papua (37.6 wt%, d.b). The measured oil content for both samples is within the 30–40 wt% oil content on a seed basis as reported in the literature (Holilah et al., 2015; Kumar et al., 2015).

The fatty acid composition of the oil as determined by using GC–MS is presented in Table 2. The fatty acid composition of the oil extracted from the seeds obtained at West Java and Papua was compared with the results from other locations (East Java and India) as reported in the literature (Holilah et al., 2015; Kumar et al., 2015). From Table 2, it can be observed that the fatty acid composition of the oil at four different locations greatly differ from one another. For instance, trisperma oil from West Java primarily consists of α-eleostearic acid (36.8%). In contrast, trisperma oil from Papua do not contain α-eleostearic acid and primarily consists of oleic acid (51.2%) whereas the oil from East Java and India mainly consists of oleic acid (30.2%) and nervonic acid (44.1%), respectively. The total saturated fatty and unsaturated fatty acid also greatly varies from 11.5–44.9% and 47–88.5%, respectively.

After the isolation of oil from the seeds, the pressed cake was mixed with the shell (cake content: 0, 10, 20 wt%) before used as a substrate for the cultivation of BSFL. The protein and lignocellulose content of the mixture (cake content of 20 wt%) were analyzed and the results are shown in Table 3. The sample from Papua had a higher protein content in comparison to the sample from West Java whereas the oil content is almost negligible because the oil had been extracted using a Soxhlet extraction with n-hexane. The sample from West Java had an oil content of 11.6 wt%, d.b. which indicates that there was a considerable amount of oil remain in the pressed cake after the isolation of oil using a hydraulic pressing machine. The sample from West Java had a higher lignin (47.9 wt%, d.b.) and lower hemicellulose content (10.1 wt%, d.b.) when compared to the sample from Papua (29.9 and 39.2 wt%, d.b., respectively).

Table 3
Protein, oil and lignocellulose composition of the remaining biomass sample from West Java and Papua (cake content of 20 wt%).

Component	Percentage (wt%, d.b.)	
	West Java	Papua
Protein	10.7	19.1
Oil	11.6	< 1
Hemicellulose	10.1	39.2
Cellulose	27.5	10.9
Lignin	47.9	29.9
Ash	n/a	0.2

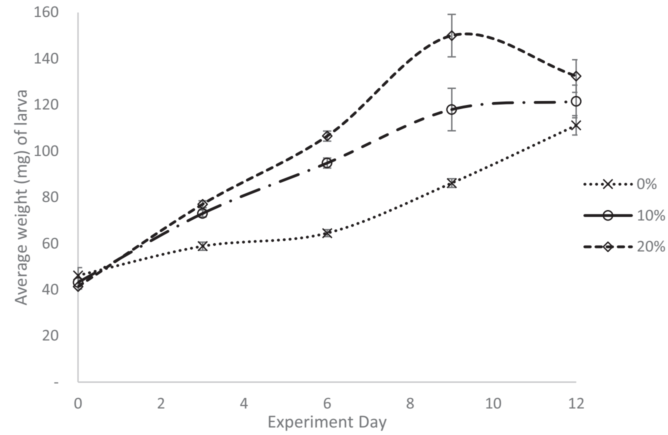

Fig. 1. Weight profile of larvae fed at different cake content (moisture content: 60 wt%, feeding rate: 75 mg/larvae/d, substrate depth: 10 cm, dark condition).

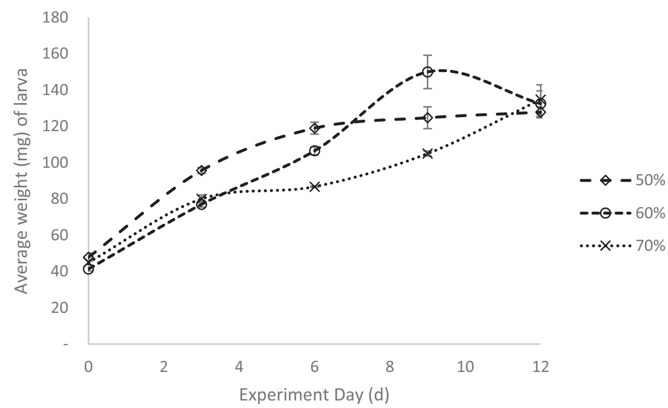

Fig. 2. Weight profile of larvae fed at different moisture content (cake content: 20 wt%, feeding rate: 75 mg/larvae/d, substrate depth: 10 cm, dark condition).

Valorization of remaining biomass from trisperma seeds as a substrate for the cultivation of BSFL

The effect of cake and moisture content on the growth of BSFL had been studied and the results are shown in Fig. 1-2. Fig. 1 shows the weight profile of larvae at different cake content. Based on the ANNOVA (level of significance: 95%), the effect of cake content (0, 10, 20 wt%) is significantly different among different experiment day (*P*-value < .0001). However, the effect of cake content is not significantly different at day 0 (P-value of 0.098) and day 12 (P-value of 0.123). From Fig. 1, it can be observed that initially the average weight of the larvae was approximately 41–46 mg/larvae after fed with chicken meal for 7 d. At experiment day 0, the chicken meal was replaced with the trisperma biomass (moisture content of 60 wt%, feeding rate of 75 mg/larvae/d) and the weight of larvae in the rearing container (dark condition) was continuously monitored. From the figure, it can be observed that the average growth rate of the larvae varies with the concentration of the cake.

At a cake content of 20 wt%, the average weight of the larvae continuously increased from approximately 41.3 ± 1.0 to 150 ± 9.2 mg after 9 d before slightly decreased to 132.5 ± 7.1 mg when continuously fed with the substrate for 12 d. A slower larval growth rate was observed at lower cake content. After 12 d of feeding at similar conditions, the average weight of larvae at a cake content of 0 and 10 wt% were approximately 111.2 ± 4.2 and 121.5 ± 2.8 mg, respectively. A faster growth rate and production of biomass at higher cake content are due to higher protein availability in the feed. At a cake content of 20 wt%, the protein content in the feed was 10.7 wt% which was higher than the protein content in the feed for cake content of 10 and 0 wt% (8 and 5.2 wt%, respectively).

Fig. 2 shows the weight profile of larvae at different moisture content. Based on the ANNOVA (level of significance: 95%), the effect of moisture content (50, 60, 70 wt%) is significantly different among different experiment day (*P*-value < .0001). However, the effect of moisture content is not significantly different at day 0 (P-value of 0.014) and day 12 (P-value of 0.363). From Fig. 2, it can be observed that for a moisture content of 50 wt%, the larval growth was faster during the first 6 d before it started slowing down and reached its plateau (124.8 ± 6.0 mg) after 9 d and slightly decreased when continuously fed with the substrate for 12 d. When the moisture content of the substrate increased to 60 wt%, the average weight reached its plateau (150 ± 9.2 mg) after 9 d before slightly decreased when continuously fed with the substrate for 12 d. As for a moisture content of 70 wt%, the average weight continue to increase from 45 ± 1.4 to 134.8 ± 8.1 mg after 12 d of treatment.

The present study showed that moisture content in the feed plays a crucial role in the growth of BSFL. These results resemble the previous findings by Fatchurochim et al. (1988) that the production rate of *H. illucens* was greatest at moisture content of 40–60 wt% while significantly less in substrate containing 70 wt% moisture. Cammark and Tomberlin (2017) demonstrated that larvae reared with substrate containing 70 wt% moisture developed faster, grew larger and required less food than those reared with substrate containing 55 wt% moisture. Other studies also reported that consumption of substrate with low moisture content could significantly reduce relative growth rate and conversion of digested food (Scriber, 1977; Reese and Beck, 1978; Martin and Van't Hof, 1988; Schmidt and Reese, 1988; Timmins et al., 1988; Rath, 2010).

Fig. 3 shows the weight profile of larvae at two different lighting conditions viz. i) dark condition imply that the rearing container was covered by a black plastic cover, ii) light condition imply that the rearing container was covered by a transparent plastic cover. Based on the ANNOVA (level of significance: 95%), the effect of light conditions (light and dark) is significantly different among different experiment day (*P*-value < .0001). However, the effect of light condition is not significantly different at day 0 (P-value of 0.067), day 9 (0.41) and day 12 (P-value of 0.287).

From Fig. 3, it can be noted that at light condition, the average weight of the larvae reached its plateau (129.5 ± 13.4 mg) after 9 d before slightly decreased when continuously fed with the substrate for 12 d. This value was lower in comparison when BSFL were reared in a dark condition in which the average weight of the larvae reached its plateau (150 ± 9.2 mg) after 9 d. When the larvae were subjected to light condition, the larvae received a higher light intensity in comparison to the light intensity received under dark condition. As a result, the average weight of the larvae after 9 d decreased from 150 ± 9.2 mg to 129.5 ± 13.4 mg. This is in line with the results obtained by Holmes et al. (2018) that development time of black soldier fly larvae decreased with an increased in light intensity and relative humidity.

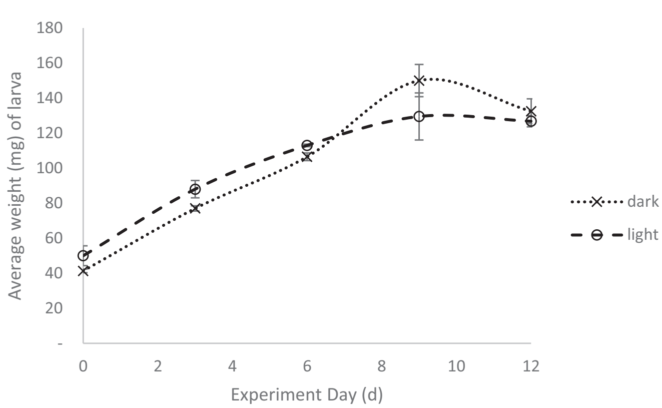

Fig. 3. Weight profile of larvae fed at light and dark conditions (cake content: 20 wt%, moisture content: 60 wt%, feeding rate: 75 mg/larvae/d, substrate depth: 10 cm).

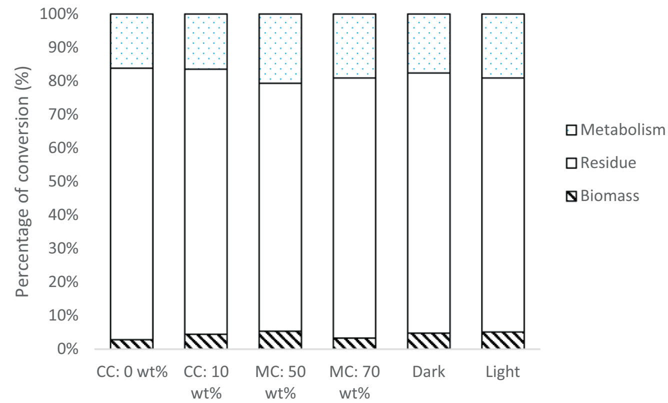

Fig. 4. The relative proportion of feed at different cake content, moisture content and light condition that was converted into biomass (prepupal weight), used for metabolisms and remained as residuals.

Fig. 4 shows the relative proportion of feed that was converted into prepupal biomass, residual matter and used for metabolism (calculated by mass balance). The results showed that approximately 2.8–5.4 wt%, d.b. of the feed was transformed into prepupal biomass. Almost 73.9–81% of the feed remained as residue which comprises of feces excreted by the larvae or feed that had not been consumed by the larvae. Based on the proportion of feed that was converted into prepupal biomass and remained as residue, the proportion of feed that was used by the larvae for their metabolic needs lies in the range of 16.2–20.7%.

Highest conversion percentage of feed into biomass (5.4%) was noted at a moisture content of 50 wt% (cake content: 20 wt%, feeding rate: 75 mg/larvae/d, dark condition) whereas the lowest value (2.8%) was observed at a cake content of 0 wt% (moisture content: 60 wt%, feeding rate: 75 mg/larvae/d, dark condition). As such indicates that the amount of available protein in the feed is influential in the bioconversion of the feed into preppupal biomass. The amount of feed that was converted into prepupal biomass obtained in this study is slightly lower than the results presented Diener et al. (2009); around 6–16.1% of chicken feed was transformed by BSFL into prepupal biomass. As such may be due to the ability of BSFL to digest different type of organic matter which depends on the composition of material, microbial symbionts and digestive enzymes (Zhou et al., 2013).

The ability and effectiveness of BSFL in digesting the trisperma biomass can be quantified by calculating the assimilation efficiency (AE). From Table 4, it can be observed that the assimilation efficiency varies from 19 to 26.1% Moisture content plays an important role in the ability of the BSFL to digest the feed. Diener et al. (2009) reported that that the optimum moisture content for BSFL feed was around 60 wt% which explains why the value of AE decreased from 26.1 to 21.1 wt% when the value of moisture content increased from 50 to 70 wt%. A lower cake content in the substrate also results in a lower value of AE due to lower protein content. Cammack and Toberlin (2017) emphasized that larvae reared on balanced diet which contains 21% protein and 21% carbohydrate developed faster with the least amount of food and accordingly results in a higher value of AE.

The assimilation efficiency obtained in this study resembles the results obtained in previous studies. Manurung et al. (2016), reported that the AE of BSFL when fed with trisperma seeds was 27.7% whereas according to Abduh et al. (2017), the AE of BSFL when fed with rubber seeds was in the range of 23 to 29%. In another study, Tarigan et al. (2018) reported that when BSFL was fed with a mixture of tofu residue

Table 4
Estimation of conversion of digested feed, assimilation efficiency and productivity of prepupae fed with trisperma biomass from West Java at different conditions.

Cultivation condition	Assimilation efficiency (%)	Waste reduction index (−)	Efficiency of conversion of digested feed (%)	Productivity (g/m^2/d)
Cake content: 0 wt%	19.0	1.3	11.5	12.6
Cake content: 10 wt%	20.9	1.7	14.5	17.6
Moisture content: 50 wt%	26.1	2.9	14.5	21.9
Moisture content: 70 wt%	21.1	1.8	14.3	17.4
Dark	24.2	2.7	20.3	28.5
Light	24.3	2.7	15.0	21.0

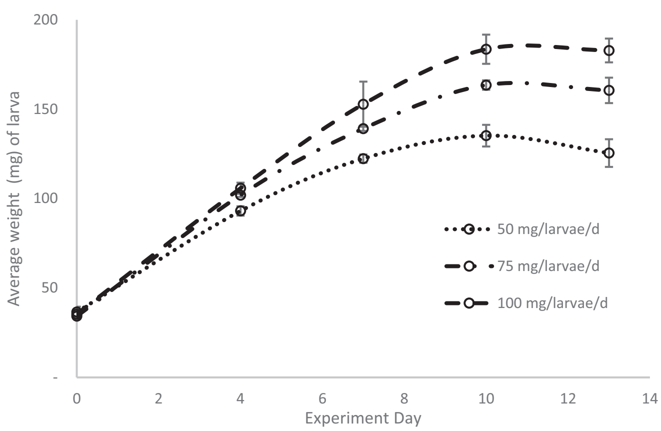

Fig. 5. Weight profile of larvae fed at different feeding rate (dark condition, cake content: 20 wt%, moisture content: 60 wt%).

and rice straw, the value of AE lies in the range of 25.7 to 39.9%. Although insects are much less efficient in assimilation as compared to birds and carnivores, the unassimilated biomass that remains as residue or passes out feces is important to the decomposer-based trophic system (Price, 1999). It is reported in the literature that > 50% of the ingested material is not assimilated. This residue or feces is abundant than what produced by other vertebrates in the same area and consequently may be further valorised to produce other bioproducts such as compost (Price, 1999, Tarigan et al., 2018).

Waste reduction index (WRI) can be calculated by dividing the value of AE with the time that the larvae transformed into prepupae (9–15 d); the WRI values are in the range of 1.3–2.9. These results are well within the range of WRI reported in the literature. According to Abduh et al. (2017), the WRI of BSFL when fed with rubber seeds was in the range of 2.2 to 2.6 whereas Diener et al. (2009) reported that the WRI of BSFL when fed with chicken feed was in the range of 1.1 to 3.8. In another study by Tarigan et al. (2018), the calculated WRI lies in the range of 1.3 to 2.4 when BSFL was fed with a mixture of tofu residue and rice straw.

The efficiency of BSFL in converting the feed into its biomass can be estimated from the ECD value which is the ratio of dry weight of prepupae to the difference between initial dry weight of feed and dry weight of residue. From Table 4, it can be observed that the ECD values vary from 11.5 to 20.3%. The productivity of the prepupae at different conditions was estimated from the dry weight of the prepupae divided by the area of the container and period of cultivation and the results are shown in Table 4. Given that the rearing container used in this study has an area of 0.0026 m^2 (every container has 20 larvae) and the period of cultivation was 9 to 15 d, the productivity of the prepupae lies in the range of 12.6 to 28.5 g/m^2/d.

Based on the results of the experiments, the highest productivity of prepupal biomass (28.5 g/m^2/d) was obtained when the BSFL was reared in a dark condition with a cake content of 20 wt% and moisture content of 60 wt%. The lignocellulose composition as well as oil and protein content of the residue which comprises the excretory products and unconsumed feed was analyzed and the results are compared with the lignocellulose composition of the feed. The hemicellulose content decreased from 10.1 to 9.5 wt%, d.b. whereas the cellulose content decreased from 27.5 to 23.4 wt%, d.b. The lignin content increased from approximately 47.9 to 51 wt%, d.b. which indicates that BSFL was not able to digest lignin during the bioconversion process.

These findings are in agreement with the results obtained by Li et al. (2011) and Zheng et al. (2012) that BSFL has the ability to digest hemicellulose and cellulose but not lignin. The oil content decreased from 11.6 wt%, d.b. in the feed to 1.0 wt%, d.b. in the residue. The protein content also decreased from 10.7 wt%, d.b. in the feed to 7.0 wt %, d.b. in the residue. As such indicates that the fat and protein in the feed was utilized for biomass accumulation of the BSFL during the bioconversion process.

Effect of feeding rate on the cultivation of BSFL

This section studied the effect of feeding rate on the cultivation of BSFL fed with trisperma seeds from West Java. The feeding rate was varied (50, 75, 100 mg/larvae/d) whereas the other cultivation conditions were set constant at the conditions that provide the highest productivity of prepupal biomass (cake content of 20%, moisture content of 60 wt%, substrate depth of 10 cm, dark condition).

Fig. 5 shows the weight profile of larvae at different feeding rates. Based on the ANNOVA (level of significance: 95%), the effect of feeding rate (50, 75, 100 mg/larvae/d) is significantly different among different experiment day (P-value $< .0001$). However, the effect of feeding rate is not significantly different at day 0 (P-value of 0.43). From Fig. 5, it can be observed that initially the average weight of the larvae was approximately 35.4 mg after fed with chicken meal for 7 d. At day 0, the chicken meal was replaced with the trisperma biomass (cake content of 20%, moisture content of 60%) and the weight of larvae in the rearing container (dark condition) was continuously monitored. From the figure, it can be observed that the average growth rate of the larvae varies with feeding rate. At a feeding rate of 50 mg/larvae/d, the average weight of the larvae continuously increased from approximately 35.4 to 135.21 mg after 10 d before slightly decreased to 125.5 mg when continuously fed with the substrate for 13 d.

A similar profile was observed when BSFL was fed at a feeding rate of 75 mg/larvae/d. The average weight of the larvae reached its plateau (163.5 mg) after 10 d before slightly decreased to 160.5 mg when continuously fed with the substrate for 13 d. As for BSFL fed at 100 mg/larvae/d, the average weight of the larvae continuously increased from 35.4 to 183.6 mg after 10 d before decreased to 182.3 mg when continuously fed with the substrate for 13 d. Such profile was due to the behaviour of the BSFL that continuously feed until they have obtained the amount of energy required to perform pupal development.

Fig. 6 shows the relative proportion of feed that was converted into prepupal biomass, used for metabolism of the larvae and remained as residual matter. The results showed that approximately 4.4–6.0% of the feed was transformed into prepupal biomass. Almost 72.3–78.8% of the feed remained as residue which comprises of feces excreted by the larvae or feed that had not been consumed by the larvae. Based on the proportion of feed that was converted into prepupal biomass and remained as residue, the proportion of feed that was used by the larvae for their metabolic needs lies in the range of 16.8–21.7%.

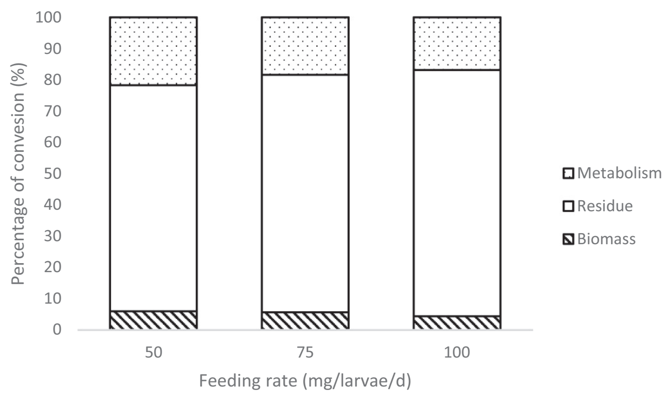

Fig. 6. The relative proportion of feed at different feeding rate (cake content: 20 wt%, moisture content: 60 wt%) converted into biomass (prepupal weight), used for metabolisms and remained as residuals.

Table 5
Estimation of conversion of digested feed, assimilation efficiency and productivity of prepupae fed trisperma biomass from West Java (cake content: 20 wt%, moisture content: 60 wt%).

Feeding rate (mg/larvae/d)	Assimilation efficiency (%)	Waste reduction index (−)	Efficiency of conversion of digested feed (%)	Productivity ($g/m^2/d$)
50	27.7	2.77	21.6	27.8
75	24.1	2.41	23.6	36.1
100	21.2	2.12	20.8	41.1

From Table 5, it can be observed that the value of AE decreased from 27.7 to 21.2% as the feeding rate was increased from 50 to 100 mg/larvae/d, and indicates that the ability of BSFL to digest all the feed provided decreased as the feeding rate increased. The WRI was calculated by dividing the value of AE with the development time of 10 d. Similarly to the values of AE, the WRI decreased as the feeding increased from 50 to 100 mg/larvae/d. From Table 5, it can be observed that the ECD increased from 21.6 to 23.6% as the feeding rate increased from 50 to 75 mg/larvae/d. Further increasing the feeding rate from 75 to 100 mg/larvae decreased the ECD to 20.8%. According to Abduh et al. (2017), the ECD of BSFL when fed with rubber seeds was in the range of 13 to 29% whereas Diener et al. (2009) reported that the ECD of BSFL when fed with chicken feed was in the range of 24 to 38%. In another study by Tarigan et al. (2018), the calculated ECD lies in the range of 12 to 22% when BSFL was fed with a mixture of tofu residue and rice straw.

The productivity of the prepupae at different feeding rates was estimated from the dry weight of the prepupae divided by the area of the container and treatment period and the results are shown in Table 6. Given that the rearing container used in this study had an area of 0.0026 m^2 (every container has 20 larvae) and the experiment day was 10 d, the productivity of the prepupae lies in the range of 27.8 to 41.1 $g/m^2/d$. Higher productivity was observed at higher feeding rates are most probably due to higher amount of protein available to be uptake by the BSFL. Nevertheless, this study is still in a laboratory scale and the results would be different if it is to be applied in an industrial scale.

Table 6
Effect of larval height on the productivity of prepupae fed with trisperma biomass (cake content: 20 wt%, moisture content: 60 wt%, feeding rate: 100 mg/larvae/d, dark condition).

Larval height (cm)	Larval initial weight (g)	Larval final weight (g)	Productivity (wet basis, $g/m^2/d$)	Productivity (dry basis, $g/m^2/d$)	Larval density ($larva/cm^2$)
4	7.6	89.2	217.9	78.9	1.4
6	10.2	137.9	341.1	123.4	2.2
8	11.8	119.5	287.7	104.1	1.9

According to Abduh et al. (2017), the productivity of the prepupae when fed with rubber seeds and reared in 6.8 × 6.8 cm (5 $larvae/cm^2$) containers was in the range of 37 to 124 $g/m^2/d$. In another study by Tarigan et al. (2018), the estimated productivity lies in the range of 73 to 180 $g/m^2/d$ when BSFL was fed with a mixture of tofu residue and rice straw and reared in 25 × 20 cm container (5 $larvae/cm^2$). Diener et al. (2009) has reported that the productivity of the prepupae was 145 $g/m^2/d$ when fed with chicken feed and reared in 3 × 2 m containers (5 $larvae/cm^2$). Possible reasons for these differences are due to the different type of feed and larval density used in these studies. The three previous studies applied a larval density of 5 $larva/cm^2$ whereas the larval density applied in this study was lower (approximately 1 $larva/cm^2$). As such indicates that increasing the larval density may possibly increase the productivity of the biomass.

Effect of substrate depth on the productivity of prepupal biomass

This section investigated the effect of substrate depth on the productivity of prepupal biomass when BSFL was fed with the remaining biomass of trisperma seeds from West Java. The substrate depth was varied between 4 and 8 cm whereas the other cultivation conditions were set constant at the conditions that provide the highest productivity of prepupal biomass (cake content of 20 wt%, moisture content of 60 wt %, feeding rate: 100 mg/larvae/d, dark condition). The results are shown in Table 7.

From Table 6, it can be noted that the larval productivity of the prepupal biomass increased from 78.9 to 123.4 $g/m^2/d$ when the substrate depth in the rearing container was increased from 4 to 6 cm. However, when the substrate depth was increased further to 8 cm, the productivity decreased to 104 $g/m^2/d$. In the previous section, the substrate depth was set at 10 cm and the productivity of prepupal biomass at similar conditions was even lower (41.1 $g/m^2/d$). As such indicates that there is an optimum substrate depth in the rearing container. Most probably if the substrate depth is too large, an anaerobic condition may prevail and hinder the growth of BSFL.

Table 6 also shows the larval density which was calculated from the amount of larvae inside the rearing container divided by the area of the rearing container (26 × 16 cm). The amount of larvae was estimated from the final weight of the larvae divided by the average weight of a larva (152 mg). The highest larval density of 2.2 $larvae/cm^2$ was obtained at a larval height of 6 cm. This value is higher than the larval density at larval height of 4 cm (1.4 $larvae/cm^2$) and 8 cm (1.9 $larvae/cm^2$). In the previous section, the larval density was approximately 1 $larvae/cm^2$ (larval height of 10 cm). These results support the previous argument that increasing the larval density may increase the productivity of prepupal biomass.

Composition of protein and oil in the prepupal biomass

The prepupae produced from the cultivation of BSFL fed with the remaining biomass of trisperma seeds obtained from West Java were analyzed to determine the protein and oil content and the results are

Table 7
Composition of protein and oil in the prepupae produced from BSFL fed with the remaining biomass of trisperma seeds obtained from West Java.

Composition	Feed	Prepupae	Diener et al. (2009)
Protein (wt%, d.b.)	10.7	45.1	37.3–42.1
Oil (wt%, d.b.)	11.6	26.6	33.1–34.2

Table 8
Fatty acid compositions of BSFL oil.

Fatty acid	BSFL oil[a]	BSFL oil[b]
Saturated fatty acid		
Capric acid (10:0)		3.8
Lauric acid (12:0)		27.8
Myristic acid (14:0)		8.1
Palmitic acid (16:0)	10	14.2
Pentadecanoic acid (15:0)		1.5
Heptadecanoic acid (17:0)		0.8
Nonadecanoic acid (19:0)		1.7
Stearic acid (18:0)	`	7.6
Arachidic acid (20:0)	2.5	
Unsaturated fatty acid		
Palmitoleic acid (16:1)		4.1
Oleic acid (18:1)	11.0	22.5
Linoleic acid (18:2)	14.6	1.8
Linoleic acid (18:3)		2.1
α-eleostearic acid (18:3)	53	

[a] This study.
[b] Zheng et al. (2012).

shown in Table 7. For the prepupae produced from the cultivation of BSFL fed with the remaining biomass of trisperma seed from West Java, the protein content in the prepupae is 45.1 wt%, d.b., 321% higher than the oil content in the feed whereas the oil content in the prepupae is 26.6 wt%, d.b., 129% higher than the oil content in the feed. This value lies in the range of oil content reported in the literature: 19–34 wt% (Diener et al., 2009; Abduh et al., 2017). The protein and oil content obtained in this study also satisfies Indonesian National Standard for protein (SNI 01-3929-2006) and fat (SNI 01-3909-2006) requirement of animal feed for broiler chickens (13.5 and 7 wt%, d.b.) Hence, the prepupae of BSFL reared on the remaining biomass of trisperma seed may find suitable application in the feed industry.

The oil in the prepupae produced from the BSFL cultivation fed by the remaining biomass of trisperma seed from West Java was extracted using Soxhlet extraction. The fatty acid composition of the oil was determined by GC–MS and the results are shown in Table 8. The composition is different when compared with the fatty acid composition of BSFL oil reported by Zheng et al. (2012). These differences are due to the different substrate used for the cultivation of the BSFL. Rice straw was used by Zheng et al. (2012) as a substrate for cultivation of BSFL whereas this study used trisperma seeds. The BSFL oil obtained in this study consists of palmitic acid (10%), stearic acid (8.9%), arachidic acid (2.5%), oleic acid (11%), linoleic acid (14.6%) and α-eleostearic acid (53%).

This composition is relatively similar with the fatty acid composition of trisperma oil obtained from West Java (Table 2) which comprises of palmitic acid (15.2%), stearic acid (6.6%), oleic acid (18.4%), linoleic acid (23.1%) and α-eleostearic acid (36.8%). The main difference is the presence of arachidic acid in BSFL oil but absent in the trisperma oil. The overall composition of palmitic acid, oleic acid and linoleic in the BSFL oil decreased in comparison to its relative composition in the trisperma oil. In contrast, the composition of α-eleostearic acid increased significantly from 36.8 to 53%. As such highlights the role of BSFL as a biological converter during the bioconversion process (Li et al., 2011; Li et al., 2015).

Conclusions and outlook

A systematic study on the use of *Reutealis trisperma* seeds as a substrate for the cultivation of black soldier fly larvae has been investigated. The characteristics of *Reutealis trisperma* seeds from two different locations particularly from Papua and West Java have been determined. The seeds have a relatively high oil (37.6–39.2 wt%, d.b.) and protein content (14.9–28.2 wt%, d.b.). The highest prepupal biomass productivity of 123.4 g/m^2/d was obtained at 20 wt% cake content, 60 wt% moisture content, 100 mg/larvae/d, dark, and 6 cm substrate depth. The protein and oil content of the biomass have been determined to evaluate the effect of *Reutealis trisperma* seeds as a substrate for the cultivation of black soldier fly larvae to produce protein and oil-rich biomass. The oil content in the biomass has been extracted and the fatty acid composition has been identified. The prepupal biomass has a relatively high amount of protein (45 wt%, d.b.) and oil content (26.6 wt%, d.b.) The prepupae may find application as a high protein feed whereas the oil extracted from the black soldier fly larvae may be utilized as a low cost biodiesel feedstock.

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